16th ISNI Congress 2023

BOOK OF ABSTRACTS

INTERNATIONAL SOCIETY OF NEUROIMMUNOLOGY

August 20

ECTRIMS
EUROPEAN COMMITTEE FOR TREATMENT AND RESEARCH IN MULTIPLE SCLEROSIS

Sponsored by ECTRIMS

Day 1 | GSNI Pre-Congress Course

Morning Session

Chairs: Roberto Furlan *(ESNI/ECTRIMS)* & V. Wee Yong *(ASNI)*

8:30am-8:45am — **Opening Remarks**
V. Wee Yong (Canada), Gianvito Martino (Italy) & Roberto Furlan (Italy)

8:45am-9:30am — **How the brain controls peripheral immune responses in health and disease**
Asya Rolls (Technion, Israel Institute of Technology - Haifa, Israel – ESNI)

9:30am-10:15am — **Clinical research in Neuroimmunology**
Fabienne Brilot (University of Sydney, Sydney, Australia – APSNI)

10:15am-10:45am — *Coffee Break*

Morning session continues

Chairs: Tika Benveniste *(ASNI)* & Qiang Liu *(APSNI)*

10:45am-11:30am — **Neuroimmunology from MRI-neuropathology transcriptomics to human organoids**
Martina Absinta (Vita-Salute San Raffaele University, Milan, Italy – ESNI)

11:30am-12:15pm — **Neuroimmunology of mood disorders**
Caroline Menard (Université Laval, Quebec City, Canada – ASNI)

12:15pm-1:15pm — *Lunch Break*

Afternoon Session

Chairs: Judith Greer *(APSNI)* & Lucas Schirmer *(ESNI/ECTRIMS)*

1:15pm-2:00pm — **Transcriptomic discoveries for Neuroimmunology**

*Florent Ginhoux (Singapore Immunology Network (SIgN) Agency for Science, Technology and Research (A*STAR), Singapore, Singapore – APSNI)*

2:00pm-2:45pm **Neuroimmunology of axonal regeneration**

Zhigang He (Harvard Medical School, Boston, USA – ASNI)

2:45pm-3:15pm *Coffee Break*

Afternoon Session continues

Chairs: Shalina Ousman *(ASNI)* & Roland Liblau *(ESNI/ECTRIMS)*

3:15pm-4:00pm **Neuroimmunology of myelination and remyelination**

Veronique Miron (University of Toronto/University of Edinburgh, Torono, Canada/Edinburgh, UK – ASNI)

4:00pm-4:45pm **Microglia – a field at its crossroads**

Rosa Paolicelli (University of Lausanne, Lausanne, Switzerland – ESNI)

August 21

Day 2 | ISNI Congress

Welcome & Introduction

8:00am-8:15am **Welcome Remarks**
Luc Vallières (Canada) & LOC & ISNI President Francisco Quintana (USA)

8:15am-8:30am **Introduction: current challenges in Neuroimmunology**
Jack Antel (McGill University - Montreal, Canada)

Plenary Session

Neuroimune interactions in neural development, cognition, and homeostasis
Chairs: Oleg Butovsky *(USA)* & Serge Rivest *(Canada)*

8:30am-9:00am **Microglia functions from development to neurodegeneration**
*Florent Ginhoux (Singapore Immunology Network (SIgN) Agency for Science, Technology and Research (A*STAR) - Singapore, Singapore)*

9:00am-9:30am **Immunoception: central representation and regulation of immunity**
Asya Rolls (Technion, Israel Institute of Technology - Haifa, Israel)

9:30am-10:00am **Unexpected interactions of the immune and nervous system - relevance for disease**
Frauke Zipp (University Mainz - Mainz, Germany)

10:00am-10:30am *Coffee Break*

Morning Breakout Sessions

Inflammation in neurodegenerative diseases
Chairs: Li Gan *(USA)* & Serge Rivest *(Canada)*

10:30am-10:48am **Gut neuroimmune interaction impacts brain synucleinopathy in Parkinson's disease models**
Soyon Hong (University College London - London, UK)

10:45am-11:06am **Decoding chronic inflammation in multiple sclerosis**
Martina Absinta (Vita-Salute San Raffaele University - Milan, Italy)

11:06am-11:24am **#137 The contribution of inflammatory astrocytes to BBB impairments in a brain-chip model of Parkinson's disease**
Aurélie de Rus Jacquet (Centre de Recherche du CHU de Québec-Université Laval – Quebec, Canada)

11:24am-11:42am **#154 Meningeal Lymphatics dysfunction disturbs cortical E/I balance**
Kyungdeok Kim (Washington University in St. Louis - St.Louis (MO), USA)

11:42am-12:00pm **#51 A CD8 T cell signature in amyotrophic lateral sclerosis 4**
Laura Campisi (Washington University School of Medicine – St. Louis (MO) - USA)

Autoantibodies in neurological conditions

Chairs: Luc Valliéres *(Canada)* & Roland Liblau *(France)*

10:30am-10:48am **The B cell immunology underlying autoantibody-mediated CNS diseases**
Sarosh Irani (Oxford University - Oxford, UK)

10:48am-11:06am **Autoimmune encephalitis and dementia – new disease concepts**
Harald Prüss (Universitätsmedizin Berlin - Berlin, Germany)

11:06am-11:24am **The cow conundrum – Novel insights into antibody-mediated disease mechanisms in multiple sclerosis**
Stefanie Kuerten (University of Bonn - Bonn, Germany)

11:24am-11:42am **#197 The emerging spectrum of foetal acetylcholine receptor antibody-related disorders (FARAD)**
Angela Vincent (Oxford, UK)

11:42am-12:00pm **#276 Anti-Ganglioside (Complex) Antibodies In Patients From The International Guillain-Barré Syndrome Outcome Study**
Robin C. M. Thomma (Rotterdam, the Netherlands)

Sponsored Lunch Break Symposium by EMD Serono

12:00pm-12:15pm *Get your lunch*

Evolving Concepts in Multiple Sclerosis Disease Progression: The impact of peripheral and centrally-driven neuroinflammation

12:15pm-12:20pm **Welcome**
Dr. V. Wee Yong

12:20pm-12:30pm **Neuropathological mechanisms in peripherally and centrally-derived inflammation in MS**
Dr. V. Wee Yong

12:30pm-12:40pm **The evolving clinical course of MS and its impact on disability progression**
Dr. Jiwon Oh

12:40pm-12:50pm **Emerging fluid biomarkers of MS disease progression**
Dr. Raphael Schneider

12:50pm-1:00pm **Audience Q&A**
Dr. V. Wee Yong & Faculty

Afternoon Breakout Sessions

Non-MS autoimmune diseases

Chairs: Stefanie Kuerten *(Germany)* & Raphael Schneider *(Canada)*

1:00pm-1:15pm **Waking up to the immunology of Narcolepsy type 1**
Roland Liblau (Université Toulouse III - Toulouse, France)

1:15pm-1:30pm **Mechanisms governing autoantibody-mediated pathology in autoimmune neurologic diseases.**
Kevin O'Connor (Yale School of Medicine - New Haven (CT), USA)

1:30pm-1:45pm **Immunopathology of NMOSD and MOGAD**
Kazuo Fujihara (Southern TOHOKU Research Institute for Neuroscience - Fukushima, Japan)

1:45pm-2:00pm **#42 Autoantibody effector functions in MOGAD**
Soumya S. Yandamuri (Yale School of Medicine, Yale University – New Haven (CT), USA)

2:00pm-2:15pm **#337 Regulation of Th17 and B cells by interferon-gamma in a novel Neuromyelitis Optica Disease Spectrum Disorder animal model.**
Gabriel Arellano (Northwestern University – Evanston (IL), USA)

2:15pm-2:30pm **#251 Pathogenic complement activation in myasthenia gravis depends on affinity matured autoantibodies with synergistic subunit specificities**
Sebastian Holdermann (University Hospital Basel and University of Basel - Basel, Switzerland)

2:30pm-2:45pm **#261 Single-cell phenotyping of B cells to investigate Rituximab response in patients with Myasthenia Gravis**
Jacopo Morroni (Fondazione Policlinico Universitario "A. Gemelli" IRCCS – Rome, Italy)

2:45pm-3:00pm **#3 The emerging role of autoreactive T cells in Guillain-Barré syndrome**
Lenka Súkeníková (ETH - Zurich, Switzerland)

Psychoneuroimmunology

Chairs: Jessica Deslauriers *(Canada)* & Frauke Zipp *(Germany)*

1:00pm-1:15pm **Immune-Brain Interactions in Depression**
Scott Russo (Icahn School of Medicine at Mount Sinai - New York (NY), USA)

1:15pm-1:30pm **Sex-specific brain and gut barrier alterations underlie stress vulnerability vs resilience in mice and human depression**
Caroline Menard (Université Laval - Quebec City, Canada)

1:30pm-1:45pm **Using circadian neuroimmunology to dissect molecular, cellular, and functional outcomes in health and disease**
Nader Ghasemlou (Queen's University - Kingston, Canada)

1:45pm-2:00pm **#300 The presence of antibodies against muscarinic acetylcholine receptors in people with first episode psychosis predicts a worse clinical outcome at 12 months follow-up**
Judith Greer (The University of Queensland – Brisbane, Australia)

2:00pm-2:15pm **#142 Interaction between a high fat diet and stress: effect on depression in relation to the gut microbiome-endocannabinoidome axis**
Giada Giorgini (Université Laval – Quebec, Canada)

2:15pm-2:30pm **#12 Behavioral as well as hippocampal transcriptomic and microglial responses differ across sexes in adult mouse offspring exposed to a dual genetic and environmental challenge**
Micaël Carrier (CHU de Québec-Université Laval - Québec, Canada / University of Victoria – Victoria, Canada)

2:30pm-2:45pm **#172 Psychological stress exacerbates immunoglobulin E-dependent chronic allergic skin inflammation via suppression of M2 macrophage-induced efferocytosis**
Soichiro Yoshikawa (Juntendo University Graduate School of Medicine – Tokyo, Japan)

2:45pm-3:00pm **#400 Neurovascular mitochondrial susceptibility impacts blood-brain barrier function and behavior**
Jorge Alvarez (University of Pennsylvania – Phildaelphia (PA), USA)

Learning about Neuroimmunology through MS – Sponsored by ACTRIMS

Chairs: Jacqueline Quandt *(Canada)* & Burkhard Becher *(Switzerland)*

1:00pm-1:24pm **An unexpected role of B cells in neuromyelitis optica**
Thomas Korn (Technische Universität München - Munich, Germany)

1:24pm-1:48pm **Age-sensitive immune markers in multiple sclerosis: relation to biological sex and treatment response**
Catherine Larochelle (University of Montreal - Montreal, Canada)

1:48pm-2:12pm **Decoding and targeting cell type-specific signatures underlying progressive neuroinflammation**
Lucas Schirmer (University of Heidelberg - Mannheim, Germany)

2:12pm-2:36pm **The Impact of Aging on Glia and Neuroinflammation: Implications for MS and EAE**
Benjamin Segal (The Ohio State University – Colombus (OH), USA)

2:36pm-3:00pm **Microglia in multiple sclerosis – pathogenesis and imaging**
Laura Airas (University of Turku - Turku, Finland)

ISNI Keynote Lecture

The Dale McFarlin Lecture

Chair: Jack Antel *(Canada)*

3:15pm-4:15pm **Tackling Heterogeneity in Multiple Sclerosis: On Path to Precision Neuroimmunology**
Amit Bar-Or (University of Pennsylvania (UPenn), Philadelphia, USA)

Poster Session I

4:15pm-6:15pm

Autoantibodies in neurological conditions
Immuno-neuro-oncology
Inflammation in neurodegenerative diseases
Non-MS autoimmune diseases
Psychoneuroimmunology
Biomarkers of neuroinflammation

August 22

Day 3 | ISNI Congress

Plenary Session

Pathological mechanisms in autoimmune diseases affecting the nervous system

Chairs: Christopher Power *(Canada)* & Thomas Korn *(Germany)*

8:00am-8:30am	**EBV-Mediated Molecular Mimicry in Multiple Sclerosis** *Francisco Quintana (Stanford University – Stanford (CA), USA)*
8:30am-9:00am	**B cells, BAFF and CNS inflammation** *Jen Gommerman (University of Toronto - Toronto, Canada)*
9:00am-9:30am	**Antibody Defined CNS Autoimmune Neurological Disorders: Discovery to Near Cure** *Sean Pittock (Mayo Clinic – Rochester (MN), USA)*
9:30am-10:00am	**New insights into how brain and periphery communicate** *Jonathan Kipnis (Washington University in St. Louis, School of Medicine – St. Louis (MO), USA)*
10:00am-10:30am	*Coffee Break*

Morning Breakout Sessions

Involvement of glial cells in neuroinflammation

Chairs: Nathalie Arbour *(Canada)* & Mikael Simons *(Germany)*

10:30am-10:48am	**Regulatory glial cell-cell interactions** *Francisco Quintana (Harvard Medical School, Brigham and Women's Hospital - Boston (MA), USA)*
10:48am-11:06am	**Insights from a unique model to study de novo myelination** *Zhigang He (Harvard Medical School - Boston (MA), USA)*
11:06am-11:24am	**What is the role of reactive astrocytes in disease?** *Shane Liddelow (NYU Langone Health - New York (NY), USA)*

11:24am-11:42am **#116 Interleukin-3 coordinates glial-peripheral immune crosstalk to incite multiple sclerosis**
Cameron S. McAlpine (Icahn School of Medicine at Mount Sinai – New York (NY), USA)

11:42am-12:00pm **#9 Fibulin-2 impedes oligodendrogenesis through engaging the Notch signaling pathway**
Samira Ghorbani (University of Calgary – Calgary, Canada)

Influence of sex on neuroinflammation

Chairs: Lisa Osborne *(Canada)* & Ari Waisman *(Germany)*

10:30am-10:48am **Sex differences in microglial metabolism and implications for neural development**
Staci Bilbo (Duke University - Durham (NC), USA)

10:48am-11:06am **Female sex exacerbates age-related changes in microglia**
Marina Lynch (Trinity College Dublin - Dublin, Ireland)

11:06am-11:24am **Adiposity interacts with female sex to promote T helper 1 inflammation and autoimmunity**
Shannon Dunn (University of Toronto - Toronto, Canada)

11:24am-11:42am **Biological sex as a determinant in inflammatory T cell responses in chronic CNS autoimmunity**
Manu Rangachari (Université Laval - Quebec, Canada)

11:42am-12:00pm **#305 Role of sex, gonadal hormones and dutasteride treatment on central and peripheral inflammation in a mouse model of Parkinson's disease.**
Amandine Isenbrandt (Université Laval – Quebec, Canada)

12:00pm-1:00pm *Lunch Break*

Afternoon Breakout Sessions

Microglia in neural development, remodelling, and protection

Chairs: Staci Bilbo *(USA)* & David Gosselin *(Canada)*

1:00pm-1:15pm **Diverse macrophages sense and govern brain environment**
Takahiro Masuda (Kyushu University - Fukuoka, Japan)

1:15pm-1:30pm **The many roles of microglia in the pathogenesis of neurodegeneration**
Rosa Paolicelli (University of Lausanne - Lausanne, Switzerland)

1:30pm-1:45pm **Roles of dark microglia during normal brain development**
Marie-Eve Tremblay (University of Toronto – Toronto, Canada)

1:45pm-2:00pm **Microglia are required for remyelination**
Jason Plemel (University of Edmonton – Edmonton, Canada)

2:00pm-2:15pm **#289 Avb8-Tgfb1 signaling in brain vascular and microglial development**
Gabriel L. McKinsey (University of California San Francisco – San Francisco (CA), USA)

2:15pm-2:30pm **#128 Stearoyl-CoA desaturase-1 impairs the regenerative and anti-inflammatory properties of microglia and T cells in the brain**
Jeroen F.J. Bogie (Hasselt University – Diepenbeek, Belgium)

2:30pm-2:45pm **#17 APOE4 Impairs Microglia Response to Neurodegeneration in Alzheimer's Disease**
Neta Rosenzweig (Brigham and Women's Hospital/ Harvard Medical School – Boston (MA), USA)

2:45pm-3:00pm **#83 Microglia coordinate cellular interactions during spinal cord repair in mice**
Faith H. Brennan (Queen's University - Kingston, Canada)

T cells in Neurological diseases

Chairs: Shannon Dunn *(Canada)* & Manu Rangachari *(Canada)*

1:00pm-1:15pm **Stranger Things about IL-12 and IL-23**
Burkhard Becher (University of Zurich - Zurich, Switzerland)

1:15pm-1:30pm **Th17 cells- to be, or not be, is the question before us**
Vijay Kuchroo (Harvard Medical School and Brigham and Women's Hospital - Boston (MA), USA)

1:30pm-1:45pm **IL-17 controls CNS autoimmunity through the intestinal microbiome**
Ari Waisman (University Medical Center of the Johannes Gutenberg University - Mainz, Germany)

1:45pm-2:00pm **#11 TCF1 is a determinant of homeostatic versus pathogenic Th17 cell state**
Ana C. Anderson (Brigham and Women's Hospital and Harvard Medical School – Boston (MA), USA)

2:00pm-2:15pm **#193 Tissue-resident memory T cells sustain the chronic phase of experimental autoimmune encephalomyelitis**
Frederick Masson (University Toulouse III – Toulouse, France)

2:15pm-2:30pm **#16 Brain endothelial antigen presentation detains CD8 T cells at the blood-brain barrier and contributes to its breakdown**
Javier Pareja (Theodor Kocher Institute, University of Bern – Bern, Switzerland)

2:30pm-2:45pm **#355 Impaired CNS Immunosurveillance by CCR7+ CD4 T cells during chronic neuroinflammation**
Elizaldi Sonny (University of California Davis – Davis (CA), USA)

2:45pm-3:00pm **#102 The transcription factor c-Maf promotes immunoregulation of CD8+ T cells in multiple sclerosis**
Norio Chihara (Kobe University Graduate School of Medicine – Kobe, Japan)

Biomarkers of neuroinflammation

Chairs: Catherine Larochelle *(Canada)* & Martina Absinta *(Italy)*

1:00pm-1:15pm **Compartment specific (endo)phenotyping of neuroinflammation**
Heinz Wiendl (University of Münster, Münster, Germany)

1:15pm-1:30pm **Biomarkers of relapsing course in MOG antibody-associated disease**
Fabienne Brilot (University of Sydney, Sydney, Australia)

1:30pm-1:45pm **What Information Does the Serum Biomarker Neurofilament Light Chain Offer in the Management of Multiple Sclerosis?**
Mark Freedman (University of Ottawa, Ottawa, Canada)

1:45pm-2:00pm **#59 CanProCo Study; Examining Prognostic Protein Biomarkers in Sera and Plasma from Multiple Sclerosis Patients**
Fiona Tea (CrCHUM – Montreal, Canada)

2:00pm-2:15pm **#286 Machine learning identifies a combination of immunological and clinical parameters as the best predictor of MS disease progression**
Stephanie Zandee (CRCHUM - Montreal, Canada)

2:15pm-2:30pm **#266 Biomarkers in autoimmune diseases of the central nervous system : the autoimmune encephalitis challenge**
Chloe Bost (Université Toulouse III – Toulouse, France)

2:30pm-2:45pm **#310 Interleukin-7 receptor alpha is increased on CD4+ effector T cells in clinically established multiple sclerosis**
Raphael Schneider (BARLO MS Centre – Toronto, Canada)

2:45pm-3:00pm **#319 Immunosenescence trajectories in diverse human populations: evaluation of immune age towards personalized therapies**
Hanane Touil (Columbia University – New York (NY), USA)

Learning about neuroimmunology through Parkinson's disease - Sponsored by ASAP

Chairs: John Lukens *(USA)* & Michael Heneka *(Germany)*

1:00pm-1:24pm **Parkinson's Disease-proteins regulate the transition from innate to adaptive immunity**
Michel Desjardins (University of Saskatchewan - Saskatoon, Canada)

1:24pm-1:48pm **Innate Immune Dysfunction in People with Parkinson's and Pre-clinical Models of disease**
Malu Tansey (College of Medicine University of Florida – Gainesville (FL), USA)

1:48pm-2:12pm **Unveiling the Enigma: Exploring Neuroinflammation's Impact on Parkinson's Disease**
Ted Dawson (Johns Hopkins University School of Medicine – Baltimore (MD), USA)

2:12pm-2:36pm **Microglia iron overload and ferroptosis cause neurodegeneration**
Timothy Hammond (Sanofi – Cambridge (MA) USA)

2:36pm-3:00pm **#344 Brain-to-gut trafficking of alpha-Synuclein by CD11c+ macrophages in Parkinson's Disease**
Rhonda L. McFleder (Germany)

ISNI Keynote Lecture

The Rita Levi-Montalcini Neurobiology Lecture

Chair: Alexandre Prat *(Canada)*

3:15pm-4:15pm **Inflammation and Neurodegeneration in multiple 26clerosis: A single and common pathway?**
Hans Lassmann (Medical University Wien, Vienna, Austria)

Poster Session II

4:15pm-6:15pm

Biomarkers of neuroinflammation

Influence of sex on neuroinflammation

Involvement of glial cells in neuroinflammation

Microglia in neural development, remodelling, and protection

Neuroimmunology general I

T cells in Neurological diseases

August 23

Day 4 | ISNI Congress

Plenary Session

Neuroinflammation in degenerative diseases

Chairs: Malu Tansey *(USA)* & Jean-Pierre Julien *(Canada)*

8:00am-8:30am	**TBD** *Michael Heneka (University of Bonn - Bonn, Germany)*
8:30am-9:00am	**Neuroimmune modulation by AD risk genes** *Li Gan (Weill Cornell Medicine - New York (NY), USA)*
9:00am-9:30am	**Honing in on the molecular orchestrators of neuroprotective microglial responses in Alzheimer's disease** *John R. Lukens (University of Virginia - Charlottesville (VA), USA)*
9:30am-10:00am	**Brain-Immune ecosystem and immunotherapy to defeat dementia** *Michal Schwartz (Weizmann Institute of Science - Rehovot, Israel)*
10:00am-10:30am	*Coffee Break*

Morning Breakout Sessions

CNS barriers

Chairs: Britta Engelhardt *(Switzerland)* & Jorge Alvarez *(USA)*

10:30am-10:48am	**Regulation of the blood-brain barrier in health and disease** *Richard Daneman (University of California - San Diego (CA), USA)*
10:48am-11:06am	**Ependymal cell dysregulation during neuroinflammatory events** *Jo Anne Stratton (McGill University - Montreal, Canada)*
11:06am-11:24am	**Brain endothelial cells under inflammatory challenge** *Alexandre Prat (Université de Montréal - Montreal, Canada)*

11:24am-11:42am **#243 Cerebral microangiopathy is a key mediator of interferon-alpha neurotoxicity.**
Barney Viengkhou (The University of Sydney – Sydney, Australia)

11:42am-12:00pm **#127 CRTAM-Necl2 interaction at the glia limitans triggers astrogliosis and protection against EAE**
Bieke Broux (Hasselt University – Diepenbeek, Belgium)

Infection and neuroinflammation

Chairs: Louis Flamand *(Canada)* & Catherine Larochelle *(Canada)*

10:30am-10:48am **Innate immune mechanisms in neurological disease: lessons from neuroHIV and COVID-19**
Christopher Power (University of Alberta - Edmonton, Canada)

10:48am-11:06am **Mononuclear Cell Triggers of Learning and Memory Impairment : Viral effects on Integrated Systems**
Robyn Klein (Washington University School of Medicine – St. Louis - St Louis (MO), USA)

11:06am-11:24am **B cell dynamics during murine neurotropic coronavirus infection**
Cornelia Bergmann (Cleveland Clinic Lerner College of Medicine of Case Western Reserve University - Cleveland (OH), USA)

11:24am-11:42am **#64 Trichinella spiralis promotes neuroimmune remodeling that ameliorates disease in a mouse model of multiple sclerosis**
Naomi M. Fettig (University of British Columbia – Vancouver, Canada)

11:42am-12:00pm **#330 Sepsis, complement, and microglial synaptic pruning: findings in the mouse and human brain**
Kate A. Giffin (University of Michigan – Ann Arbou (MI), USA)

NOVARTIS

12:00pm-1:00pm Sponsored Lunch Break Symposium by Novartis

12:00pm-12:15pm *Get your lunch*

Understanding the Unique Features of Anti-CD20 Monoclonal Antibodies for B-Cell Depletion in MS

12:15pm-12:20pm **Welcome**

Chair: Dr. Catherine Larochelle
Dr. Amit Bar-Or
Dr. Virginia Devonshire

Afternoon Breakout Sessions

From basic to translational and clinical Neuroimmunology: contributions from young scientists | Sponsored by JNI

Chairs: Veit Rothhammer *(Germany)*, Aakanksha Dixit *(Australia)*, Jeff Dong *(Canada)*, Naomi Habib *(Israel)*, Hedwich Kuipers *(Canada)*, Olga Rojas *(Canada)* & Michal Wheeler *(USA)*

1:00pm-1:15pm **Introduction**

1:15pm-1:30pm **The road not taken - cellular cascades in Alzheimer's and aging**
Noami Habib (Hebrew University of Jerusalem – Jerusalem, Israel)

1:30pm-1:45pm **Protective glia crosstalk in autoimmune CNS inflammation**
Lena Lößlein (Universitätsklinikum Erlangen – Erlangen, Germany)

1:45pm-2:00pm **Impaired central tolerance characterises the early emergence of AQP4-specific B cells in neuromyelitis optica spectrum disorder**
Sudarshini Ramanathan (University of Sydney – Westmead, Australia)

2:00pm-2:15pm **Immune regulation of stress**
Chao Wang (University of Toronto – Toronto, Canada)

2:15pm-2:30pm **Understanding the role of B cells in neurodegeneration**
Olga Rojas (University of Toronto – Toronto, Canada)

2:30pm-2:45pm **The neural circuits controlling sickness behavior**
Jessica Osterhout (University of Utha – Salt Lake City, USA)

2:45pm-3:00pm **Discussion**

Peripheral Neuroimmunology

Chairs: Nader Ghasemlou *(Canada)* & Phillip Popovich *(USA)*

1:00pm-1:15pm **Nociceptor neuron recognition of microbes in host defense and immunity**
Isaac Chiu (Harvard Medical School, Boston, USA)

1:15pm-1:30pm **Pain neurons control cancer immunosurveillance**
Sebastien Talbot (Queen's University - Kingston, Canada)

1:30pm-1:45pm **Neuro-immune interactions in the gut**
Nader Ghasemlou (Queen's University – Kingston (ON), Canada))

1:45pm-2:00pm **#13 Multimodal control of dendritic cell functions by nociceptors**
Pavel Hanč (Harvard Medical School – Boston (MA), USA)

B cells in Neuroinflammation

Chairs: Amit Bar-Or *(USA)* & Heinz Wiendl *(Germany)*

2:00pm-2:15pm **Plasmablasts in a model of CNS autoimmunity: from biology to therapy**
Luc Vallières (University of Laval, Quebec, Canada)

2:15pm-2:30pm **#345 T:B cell communication in ectopic lymphoid follicles in CNS autoimmunity**
Anneli Peters (LMU Hospital and BMC - Munich, Germany)

2:30pm-2:45pm **#44 T-bet+ Memory B Cells Induce Disease Relapses in Experimental Autoimmune Encephalomyelitis**
Rajiv Jain (University of Calgary, Hotchkiss Brain Institute – Calgary, Canada)

2:45pm-3:00pm **#55 B cell depletion with anti-CD20 promotes neuroprotection in a BAFF-dependent manner in mice**
Angela A. Wang (University of Toronto – Toronto, Canada)

Learning about Neuroimmunology through ALS - Sponsored by ALS Society of Quebec

Chairs: Christine Vande Velde *(Canada)* & Jean-Pierre Julien *(Canada)*

1:00pm-1:20pm **Microglia and macrophages for ALS progression**
Séverine Boillée (Sorbonne Université/Hôpital de la Pitié Salpêtrière, Paris, France)

1:20pm-1:40pm **Modulating myeloid cells in ALS by targeting α5 integrin**
Bahareh Ajami (Oregon Health & Science University, Portland, USA)

1:40pm-2:00pm **Targeting innate immune genes translation in ALS**
Jasna Kriz (Université Laval, Quebec City, Canada)

2:00pm-2:20pm **Peripheral and resident immune cells crosstalk in ALS**
Cristina Limatola (Sapienza University, Rome, Italy)

2:20pm-2:40pm **Role of endogenous retroviruses in Amyotrophic Lateral Sclerosis**
Avindra Nath (NINDS, Bethesda, USA)

2:40pm-3:00pm **Restoring glial functions at the neuromuscular synapse: a clinical target in ALS**
Richard Robitaille (Université de Montréal, Montreal, Canada)

ISNI Keynote Lecture

The Immunology Lecture

Chair: Francisco Quintana *(USA)*

3:15pm-4:15pm **Checkpoints of CNS autoimmunity: from the perspective of an autoaggressive T cell**
Alexander Flügel (Georg-August University, Göttingen, Germany)

Poster Session III

4:15pm – 6:15pm

CNS barriers

CNS-infiltrating innate immune cells

Emerging therapies for neuroinflammatory conditions

Infection and neuroinflammation

Influence of diet and microbiota on neuroinflammation

Neuroimmunology general II

Novel approaches for neuroimmunologists

August 24

Day 5 | ISNI Congress

Plenary Session

Neuroinflammation in neural injury and repair

Chairs: Susanna Rosi *(USA)* & V. Wee Yong *(Canada)*

8:00am-8:30am	**Innate immune remodeling of damaged CNS barriers** *Dorian McGavern (NINDS, Bethesda, USA)*
8:30am-9:00am	**Innate immune mechanisms in remyelination** *Mikael Simons (Technical University of Munich, Munich, Germany)*
9:00am-9:30am	**Microglia regulation of myelin health across the lifespan** *Veronique Miron (University of Toronto/University of Edinburgh, Toronto, Canada/Edinburgh, UK)*
9:30am-10:00am	**Role of microglia and IL-6 signalling following brain injury** *Jana Vukovic (The University of Queensland, Brisbane, Australia)*
10:00am-10:30am	Coffee Break

Morning Breakout Sessions

Emerging therapies for neuroinflammatory conditions

Chairs: Benjamin Segal *(USA)* & Jack Antel *(Canada)*

10:30am-10:48am	**Overcoming CNS fibrosis for neuroregeneration** *V. Wee Yong (University of Calgary, Calgary, Canada)*
10:48am-11:06am	**Xenon gas treatment to restore microglial functions in neurodegenerative diseases** *Oleg Butovsky (Brigham and Women's Hospital, Harvard Medical School, Boston, USA)*
11:06am-11:24am	**#340 Peptide-coupled RBCs as treatment for autoimmune diseases – dissecting the mechanisms of immune tolerance induction** *Vasileia Kalaitzaki (University of Zurich – Zürich, Switzerland)*

11:24am-11:42am **#156 Targeting the Inflammasome in Inflammaging**
Brianna Cyr (University of Miami – Miami (FL), USA)

11:42am-12:00pm **#299 Nasal anti-CD3 mAb induces Tregs that dampen microglial activation and treat neuroinflammatory diseases including MS, AD and ALS**
Howard Weiner (Brigham and Women's Hospital, Harvard Medical School – Boston (MA), USA)

Influence of diet and microbiota on neuroinflammation

Chairs: Jen Gommerman *(Canada)* & Takashi Yamamura *(Japan)*

10:30am-10:45am **Microbial Contributions to Neurodevelopmental Disorders**
Kathy McCoy (University of Calgary, Calgary, Canada)

10:45am-11:00am **Bringing it all together: How do genes, diet and the microbiome contribute to MS?**
Sergio Baranzini (University of California, San Francisco, USA)

11:00am-11:15am **Specificity in the dietary fiber-microbiome-immune axis regulates susceptibility to neuroinflammation**
Lisa Osborne (University of British Columbia, Vancouver, Canada)

11:15am-11:30am **#121 Ketogenic diet promotes social stress resistance and modifies microglial morphology and ultrastructure in male mice**
Fernando González Ibánez (Centre de Recherche CHU de Québec-Université Laval; University of Victoria – Quebec, Canada / Victoria, Canada)

11:30am-11:45am **#284 Identification of commensal gut microbiota signatures as predictors of clinical severity and disease progression in multiple sclerosis**
Theresa Montgomery (University of Vermont - Burlington (VT), USA)

11:45am-12:00am **#119 Regulation of the antiviral immune response by the sensory nervous system**
Anais Roger (Centre d'Immunologie de Marseille-Luminy (CIML) – Marseille, France)

12:00pm-1:00pm *Lunch Break*

Afternoon Breakout Sessions

CNS-infiltrating innate immune cells

Chairs: Shalina Ousman *(Canada)* & Soheila Karimi *(Canada)*

1:00pm-1:15pm **Brain engrafted macrophages and brain injury**
Susanna Rosi (University of California at San Francisco/Altos Labs, San Francisco, USA)

1:15pm-1:30pm **Microglia regulate intraspinal and systemic neuro-immune cross-talk after spinal cord injury**
Phillip Popovich (The Ohio State University, Columbus, USA)

1:30pm-1:45pm **Cytokines produced by tissue-infiltrating innate immune cells as mediators of pain in chronic autoimmune diseases**
Steve Lacroix (Université Laval, Quebec City, Canada)

1:45pm-2:00pm **#124 Essential cytokines driving macrophage effector phenotypes in neuroinflammation**

Clara de la Rosa (University Hospital, Ludwig-Maximilians-Universität - München, Germany)

2:00pm-2:15pm **#140 Lipid metabolism directs the phenotype of foamy phagocytes in multiple sclerosis lesions**
Jerome JA Hendriks (Hasselt university – Hasselt, Belgium)

2:15pm-2:30pm **#56 Depletion of leptomeningeal neutrophils ameliorates age-dependent grey matter demyelination**
Michelle Zuo (University of Toronto – Toronto, Canada)

Immuno-neuro-oncology

Chairs: Alexandre Prat *(Canada)* & Francisco Quintana *(USA)*

1:00pm-1:15pm **Decoding function and phenotype of brain tumor infiltrating T cells**
Michael Platten (Heidelberg University, Heidelberg, Germany)

1:15pm-1:30pm **The Role of Astrocytes in Glioblastoma Pathogenicity**
Lior Mayo (Tel Aviv University, Tel Aviv, Israel)

1:30pm-1:45pm **Spatial diversity of T cell response in malignant CNS tumors**
Dieter Henrik Heiland (Germany)

1:45pm-2:00pm **Myeloid heterogeneity and functions in brain tumors**
Dolores Hambardzumyan (USA)

2:00pm-2:15pm **#285 Antiviral T cells populate glioblastoma and originate from pre-existing brain resident memory T cells**
Sierra Kleist (Dartmouth College – Lebanon (NH), USA)

2:00pm-2:15pm **#180 Analysis of factors that limit immunogenicity of intracranial melanomas**
Katarzyna Stasiak (University of Virginia – Charlottesville (VA), USA)

Novel approaches for neuroimmunologists

Chairs: Jasna Kriz *(Canada)* & Sergio Baranzini *(USA)*

1:00pm-1:15pm **Human microglia in health and disease**
Bart Eggen (University Medical Center Groningen, Groningen, the Netherlands)

1:15pm-1:30pm **Decoding the causal chain of glial activation in Alzheimer resolves the heterogeneity of the aging brain**
Philip L. De Jager (Columbia University Irving Medical Center, New York, USA)

1:30pm-1:45pm **#126 Multidimensional single-cell analysis of meningeal inflammation and cortical microglia in progressive multiple sclerosis**
Carla Rodriguez-Mogeda (Amsterdam UMC location VUmc - Amsterdam, The Netherlands)

1:45pm-2:00pm **#41 Classifying flow cytometry data using bayesian analysis helps to distinguish ALS patients from healthy controls**
Saskia Räuber (Heinrich Heine University of Düsseldorf - Düsseldorf, Germany)

2:00pm-2:15pm **#274 Development of a Human Microglia Engraftment Model for the Study of Neurodegenerative and Neuroinflammatory Diseases**

Ashley Munie Gardner (The Jackson Laboratory – Bar Harbour (ME), USA)

2:15pm-2:30pm **#322 Comparative analysis of methods to reduce activation signature gene expression in peripheral blood mononuclear cells**
Adam MacDonald (McGill University – Montreal, Canada)

Mid-Career Award

#WeAreNeuroimmunology

Chair: Fabienne Brilot *(Australia)*

2:30pm-2:45pm **Introduction**
Fabienne Brilot

2:45pm-3:00pm **Keeping the balance: T cells, environment & disease**
Markus Kleinewietfeld (Hasselt University – Diepenbeek, Belgium)

3:00pm-3:15pm **The Dilemmas of Encephalitis**
Michael Wilson (University of California – San Francisco (CA), USA)

Closing Session

Oral Presentations

August 21

Inflammation in neurodegenerative diseases

#137 The contribution of inflammatory astrocytes to BBB impairments in a brain-chip model of Parkinson's disease

A. de Rus Jacquet[1,2,3], M. Alpaugh[1,2,9], H.L. Denis[1,2], J.L. Tancredi[3,4], M. Boutin[1], J. Decaestecker[5], C. Beauparlant[5], L. Herrmann[5], M. Saint-Pierre[1], M. Parent[6,2], A. Droit[5], S. Breton[7,8], F. Cicchetti[1,2]

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The blood-brain barrier (BBB) regulates exchanges between the central nervous system and the peripheral circulation to maintain brain homeostasis, and relies on the coordinated action of brain microvascular endothelial cells (dBMECs), pericytes and astrocytes. In Parkinson's disease (PD), a neurodegenerative disorder characterized by neuronal death and astrocyte reactivity, barrier integrity is compromised which may further contribute to disease progression. The objective of this study was to determine how astrocytes with the PD-related mutation LRRK2 G2019S affect the functionality of the BBB. To do so, we first evaluated the nature of astrocyte-related biological processes affected by the LRRK2 G2019S mutation by performing a meta-analysis of RNA-sequencing datasets and characterizing the astrocyte inflammatory profile. We then established a novel BBB-on-a-chip model using human induced pluripotent stem cells (iPSCs) with the LRRK2 G2019S mutation or gene corrected controls. This in vitro model reproduced a physiologically-relevant environment and direct cell-cell interactions between dBMECs, astrocytes and pericytes, and enabled the formation of a functional blood vessel in vitro. To determine if LRRK2 G2019S astrocytes affected the formation of a functional vessel, we exposed control dBMECS to disease astrocytes and monitored vessel formation and barrier integrity using biochemical and imaging techniques. Our results indicate that LRRK2 G2019S astrocytes are pro-inflammatory, and display a transcriptomic profile suggestive of altered angiogenic properties. This mutation altered the production of trophic factors and upregulated the release of pro-inflammatory molecules via activation of the MEK/ERK pathway. As a result of this pathological secretome, disease astrocytes prevented the formation of a functional BBB and changed the morphology of vessel-forming BMECs. These observations may be driven by a dual effect of cytokine oversecretion combined with a lack of pro-angiogenesis factors. Remarkably, morphological changes to the BBB-chip induced by PD astrocytes were comparable to those observed in post-mortem substantia nigra of people with PD. Overall, the LRRK2 G2019S mutation alters astrocyte paracrine

communication, affects the in vitro formation and maintenance of a functional BBB, and this model could be used to further characterize the molecular mechanisms leading to cerebrovascular alterations in people with PD.

Keyword: *Parkinson's disease, Astrocytes, Blood-brain barrier, Microfluidic technologies, iPSCs*

#154 Meningeal Lymphatics dysfunction disturbs cortical E/I balance

Kyungdeok Kim, Jonathan Kipnis

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Cerebrospinal Fluid (CSF), produced in the choroid plexus and circulating through the ventricular system, is drained into lymphatic vessels on the meninges and deep cervical lymph nodes (dcLN). This CSF drainage through meningeal lymphatic vessels decreases with aging and in neurodegenerative conditions like Alzheimer's disease and Parkinson's disease, which in turn, exacerbates disease progresses. However, despite its translational importance, it remains unclear how dysfunction of meningeal lymphatics can affect neuronal physiology and, ultimately, animal behaviors.

Here, we show that meningeal lymphatics dysfunction leads to a synaptic excitatory/inhibitory (E/I) imbalance, which is essential for neural computation, due to the involvement of microglia and their secretory molecules. In two model systems of meningeal lymphatics dysfunction, the inhibitory synapse number decreased without affecting excitatory synaptic features, and cognitive deficits in behavior tasks accompanied it. In the dCLN afferent vessel-ligated mouse cortex, microglial morphology was swollen and had more lysosomal contents. Single-cell RNA sequencing of the microglia reveals that expressions of genes involved in phagocytic functions and disease conditions were elevated, including C1qa, Apoe, and S100a9. The enhanced complement cascade was also observed in the CSF. Pharmacological/genetic depletion of the microglia suppresses the emergence of synaptic/behavioral phenotypes. On the other hand, the expression of IL-6, a proinflammatory cytokine, was increased out of diverse cytokines in the dcLN-ligated cortex. In the cortex of dcLN-ligated IL-6 KO mice, the inhibitory synapse number was opposed, suggesting IL-6 is involved in the processes. Consistently, Direct chronic infusion of IL-6 decreased inhibitory synapse number. These collectively suggest that dysfunctional meningeal lymphatics affect synaptic circuitry and animal behavior through affected microglia and IL-6.

Previous research highlighted that aging has detrimental effects on the function and structure of meningeal lymphatics in humans and rodents. Based on the experimental results and this knowledge, we hypothesized that dysfunctional meningeal lymphatics adversely affect synaptic dysfunction initiated by aging. To explore the potential beneficial effect of meningeal lymphatic rejuvenation, we treated AAV1-VEGFc in the aged mouse. Compared to the control virus-injected group, their cortex had enhanced inhibitory functional synapses. Furthermore, this strong inhibitory tone was accompanied by the strengthening of the overall synaptic network activity.

Through this study, we revealed the mechanism how adequate function of meningeal lymphatics on microglial homeostasis, cortical synaptic E/I balance, and cognitive behaviors. Moreover, our results show that rejuvenating meningeal lymphatic drainages through AAV1-VEGFc benefits the synaptic network, crucial for neural computation.

#51 A CD8 T cell signature in amyotrophic lateral sclerosis 4

Laura Campisi[1] Shahab Chizari[2], Jessica S.Y. Ho[3], Anastasia Gromova[4], Frederick J. Arnold[4], Lorena Mosca[5], Xueyan Mei[6], Yesai Fstkchyan[3], Denis Torre[7], Cindy Beharry[3], Marta Garcia-Forn[8], Miguel Jiménez Alcázar[9], Vladislav A. Korobeynikov[10], Jack Prazich[2], Zahi A. Fayad[6], Marcus M. Seldin[11], Silvia de Rubeis[8], Craig L. Bennett[4], Lyle W. Ostrow[12], Christian Lunetta[13], Massimo Squatrito[9], Minji Byun[14], Neil A. Shneider[15], Albert R. La Spada[4], Ivan Marazzi[16],

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Motor neuron disorders (MND) cause disability and death globally. The landscape of MND is constantly evolving and additional genes and their corresponding mutations are being discovered yearly. However, our understanding of the etiology of these diseases is still limited, hampering the development of treatments and biomarkers for early diagnosis. Interestingly, in the context of MND, more than 50 genes have been discovered and most of them are highly expressed in non-neuronal cells or play a role in regulating the immune response. This is in line with recent advances in the field of neuroimmunology that have highlighted the important function of immune cells, including T cells, in the central nervous system (CNS), at the steady-state and during disease.

By using a mouse model of MND linked to a mutation in the gene Setx, which in humans causes Amyotrophic Lateral Sclerosis 4 (ALS4, a slowly progressive juvenile form of ALS), we found that the hematopoietic system contributes to the neurodegenerative process, as wild-type bone marrow transplantation in ALS mutants

halts disease manifestation. Strikingly, a high frequency of terminally differentiated effector memory (TEMRA) CD8 T cells in the peripheral blood and in the central nervous system (CNS) of ALS4 mice and patients is associate with the disease. T cell receptor (TCR) sequencing indicates that TEMRA CD8 T cells are clonally expanded, i.e. activated in response to a specific antigen, in both humans and mice. Interestingly ALS4 associated CD8 T cells in mice mediate anti-glioma but not anti-melanoma immunity, suggesting that they are: i) specific to antigen(s) of CNS origin; ii) functional.

Our findings point toward an involvement of T cells, of likely autoimmune origin, in ALS4 pathobiology, which could influence the clinical management of patients. Our study in ALS4 has the potential to reveal an immune dysfunction common to other forms of slow progressive ALS, and to shed light on potential immune-based diagnostic markers and immune therapy for patients.

Keyword: *Motor neuron disorders, Amyotrophic Lateral Sclerosis, T cells*

Autoantibodies in neurological conditions

#197 The emerging spectrum of foetal acetylcholine receptor antibody-related disorders (FARAD)

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Myasthenia gravis is caused by antibodies to the nicotinic acetylcholine receptor (AChR) of the neuromuscular junction (NMJ). Loss of AChRs and complement mediated damage to the NMJ result in muscle weakness and fatigue. Myasthenia gravis can occur in young women and the AChR antibodies (AChR-Abs) will cross the placenta from about week 16, but normally neonatal myasthenia is transient or absent. Very rarely, the antibodies lead to reduced or even absence of fetal movements in utero. In these cases, the antibodies paralyze the baby leading to neonatal death. Injection of the maternal antibodies into pregnant mouse dams induced similar features of fixed joints and paralysis in the offspring (1) and co-injection of an antibody to mouse FcRn reduced substantially the transfer of IgG and AChR-Abs to the offspring with improved outcomes in the baby mice (2). Recently, a less severe form has been recognized in offspring of some AChR-Ab positive mothers with neonatal difficulties in breathing and swallowing and persistent difficulties in speech, swallowing, hearing and other fixed difficulties; this is now termed fetal acetylcholine receptor related-antibody disorder (FARAD (3). We examined the maternal serum AChR-Abs in mothers of children with FARAD. Routine maternal AChR antibody titers ranged from 31 to 87 nM. Using adult or fetal AChR expressing cell-based assays, adult AChR titers ranged from 50 to 400 and fetal AChR titers from 200 to 3200. In each case, reactivity with fetal AChR was greater but the ratios of fetal:adult varied widely from 2 to 128. Antibodies binding to fetal AChR were of the IgG1 subclass and induced complement factor C3b deposition on the fetal AChR-expressing cells. FARAD is now a spectrum of conditions apparently caused by AChR-Abs biased towards, but not exclusively, the fetal isoform that differs from the adult isoform only in one of its five subunits. Maternal antibodies to neuromuscular junction proteins can cause severe fetal problems in utero or transient newborn weakness but the long-term effects of FARAD are less easily to explain and need further investigation.

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Keyword: *developmental disorder, maternal antibody, myasthenia gravis, fetal acetylcholine receptor*

#276 Anti-Ganglioside (Complex) Antibodies In Patients From The International Guillain-Barré Syndrome Outcome Study

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Introduction

Guillain-Barré syndrome (GBS) is an acute immune-mediated polyradiculoneuropathy in which preceding infections elicit production of cross-reactive antibodies that target gangliosides in the peripheral nervous system. Previous studies on anti-ganglioside antibodies (AGA) primarily focused on a limited set of specificities in relatively small cohorts. Glyco-arrays provide the opportunity to explore a larger spectrum of specificities, allowing examination of antibodies against numerous ganglioside complexes and antibody reactivity patterns. In this study we investigated the presence of AGA in glyco-arrays in relation to the clinical characteristics, disease course, and outcomes in patients with GBS.

Methods

Acute-phase sera from 1414 patients with GBS included in the International Guillain-Barré syndrome Outcome Study (IGOS) and 1060 healthy and neurological controls were tested for IgM, IgG and IgA against 16 glycolipids and all possible 1:1 complexes in glyco-arrays. IGOS is a prospective multinational cohort study including patients with GBS with all severities and variants. Antibody fluorescence intensities were related to preceding infections, subtypes and the disease course.

Results

Antibodies against GM1 and GQ1b complexes had higher diagnostic accuracy than antibodies to GM1 and GQ1b alone for pure motor GBS (area under the curve [AUC]: 0.814 vs 0.739) and Miller Fisher syndrome (AUC: 0.933 vs 0.774), respectively. A regression model of 18 AGA was able to discriminate GBS from controls (AUC: 0.900). Patients clustered into seven groups with distinct serum IgG antibody reactivity patterns, which differed in the distribution of GBS variants, preceding infections, electrophysiological subtypes, and the Medical Research Council sum scores and ability to walk unaided during follow-up. Moreover, three different anti-GM1 (complex) antibody reactivity patterns were also clinically distinct. Notably, these antibodies occurred in all electrophysiological subtypes. Twelve AGA were associated with the time to regain the ability to walk unaided, with two GQ1b complexes remaining after correction for known prognostic factors.

Conclusion

Combinatorial array has added value over single arrays in the diagnosis, definition of clinically distinct patient clusters, and prognosis in GBS. Moreover, different anti-GM1 (complex) antibody reactivity patterns differ in associated clinical features and these antibodies occur in all electrophysiological subtypes of GBS.

Keyword: *Guillain-Barré syndrome, Anti-ganglioside antibodies*

Non-MS autoimmune diseases

#42 Autoantibody effector functions in MOGAD

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Myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD) is an inflammatory demyelinating central nervous system condition characterized by the presence of MOG autoantibodies. We sought to investigate whether human MOG autoantibodies are capable of cytotoxicity through multiple mechanisms. Thus, we developed high-throughput assays to assess damage to live MOG-expressing cells elicited by antibody effector functions, namely complement activity (CA), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent cellular cytotoxicity (ADCC). MOGAD patient sera effectively mediate all of these effector functions. Moreover, we found CDC and ADCP are elevated closer to relapse and all IgG subclasses are capable of cytotoxicity to MOG-expressing cells. Histopathology from a representative MOGAD case revealed congruence between lesion histology and serum CDC and ADCP, and we identified NK cells, mediators of ADCC, in the cerebrospinal fluid of relapsing MOGAD patients. Thus, MOGAD-derived autoantibodies are cytotoxic to MOG-expressing cells through multiple mechanisms and effector functions assays should be further investigated as tools for predicting relapse.

#337 Regulation of Th17 and B cells by interferon-gamma in a novel Neuromyelitis Optica Disease Spectrum Disorder animal model

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Neuromyelitis Optica Spectrum Disorder (NMOSD) is a severe autoimmune astrocytophaty of the central nervous system (CNS) mediated by Th17 and autoantibody response against the water channel protein, Aquaporin-4 (AQP4). NMOSD is characterized by an initial damage to the astrocytes, followed by demyelination and axonal loss, affecting mainly the spinal cord and optic nerves, thus, patients often suffer from a paralysis and loss of sight. The understanding of the NMOSD pathogenesis and the development

of therapies has been difficult due the lack of a consistent animal model. While the role of Interferon (IFN)-beta in the disease has been describe as pathogenic, exacerbating disease symptoms, little or nothing is known about the role IFN-gamma, and the regulation of NMOSD pathogenesis. We have found that gene expression signature from NMOSD patients, is characterized by a downregulation of IFN-gamma regulated genes. Notability, actively immunization of C57BL/6 (WT) treated with neutralizing anti-IFN-gamma antibodies, IFN-gamma knockout (IFNGKO) and IFN-gamma receptor KO (IFNRKO) mice was induced using the $AQP4_{201-220}$ peptide, displaying an ascendant paralysis starting from the tail to complete hind limb paralysis. While type-I IFN KO mice (IFNAR) develop similar disease as WT, IFN-beta treatment on WT and IFNGRKO mice, exacerbates disease symptoms, similar as in human NMOSD. CNS histological analysis of the anti-IFN-gamma treated mice, showed an increased presence of T and B cells, complement and IgG deposition, in lesion sites, associated with CNS damage. CNS Th17 profile was also increased, and the use of in vivo anti-IL17-A reduce the clinical symptoms of the disease. As expected, IFN-gamma treatment in IFNGKO mice decrease disease severity with a reduction of CNS and spleen $CD4^+$ cells. Moreover, IFN-gamma regulates the IL-6 expression by B cells in an antigen specific fashion. B-cell depletion significantly reduced the disease incidence, T cells CNS infiltration and anti-$AQP4_{201-220}$ serum antibodies, while disease in mice that lack B cell receptor activation, disease was completely abolished. Finally, using Anti-IL6R treatment, we showed that IL-6 signaling is necessary for the B cell activation and their induction of Th17 cell. Our findings enhance the understanding of NMOSD pathogenesis, showing that $AQP4_{201-220}$ tolerance is strictly regulated by IFN-gamma, and its signaling, restraining B cells pathogenicity through IL-6 signaling modulation.

Keyword: *Neuromyelitis optica spectrum disorder, Interferon-gamma, Animal model, B cells, Th17 cells*

#251 Pathogenic complement activation in myasthenia gravis depends on affinity matured autoantibodies with synergistic subunit specificities

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The leading cause for the neuromuscular autoimmune disease myasthenia gravis is antibodies targeting the acetylcholine receptor (AChR), which is located on the post-synaptic side of the neuromuscular junction (NMJ). Patients with autoantibodies against the AChR suffer from muscle weakness, fatiguability and in severe cases life-threatening respiratory failure. Different mechanisms have been reported by which antibodies interfere with the signal transmission at the NMJ, such as complement activation, receptor internalization or direct receptor blockade. To date, the two major objectives in MG are to prevent the disease and to develop novel therapies with the potential to cure it. Both of these challenges require single-cell approaches (a) to investigate the development of disease-related autoantigen-specific B cells and (b) to define characteristics of autoantibodies that constitute their pathogenicity. For this purpose, we deployed a suite of methods to isolate single AChR-specific B

cells and recombinantly express the encoded antibodies. We produced eight patient-derived monoclonal IgG antibodies, all of which are hypermutated, recognize different AChR subunits, comprise different IgG subclasses and vary in their species cross-reactivity. Despite clear AChR binding in vitro and in vivo, no single antibody interrupted signal transmission, activated complement or induced myasthenic symptoms in an animal model. However, combinations of two antibodies showed increased complement activation and resulted in severe muscle weakness when administered to live rats. We examined this synergistic effect in a live cell imaging paradigm and found that antibody combinations that target different AChR subunits mediate large cluster formation on the cell surface. This cross-linking capability of antibody combinations appears to exclusively benefit complement-mediated pathology as animals treated with a complement inhibitor did not show myasthenic symptoms. Connecting the insight about the functional properties of our antibodies with sequence analysis and clonal relationship suggests that hypermutations are associated with enhanced pathogenicity. Therefore, affinity maturation, as for example observed in an immune response to a viral infection, is crucial for the development of AChR-specific autoantibodies. Consequently, this correlation indicates that a break-down of immune tolerance mechanisms rather than alternative explanations such as molecular mimicry, is the main cause of the disease.

#261 Single-cell phenotyping of B cells to investigate Rituximab response in patients with Myasthenia Gravis

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Myasthenia gravis (MG) is a B cell-mediated autoimmune neuromuscular disorder caused by autoantibodies targeting proteins expressed at the motor endplate. In 80% of MG patients autoantibodies binding the Acetylcholine Receptor (AChR) are detected. Rituximab (RTX), a chimeric monoclonal antibody targeting the CD20 B cell surface antigen, may be an effective treatment in MG patients refractory to standard immunotherapy. However, not all AChR-MG patients respond to RTX and recent studies suggest that disease duration may influence this response.

We examined the in vitro effect of RTX on B cell subsets of healthy controls (HC) and AChR-MG patients with varying treatment outcomes and disease duration. Peripheral blood mononuclear cells were incubated with RTX + rabbit complement to induce RTX-mediated killing, and cells were analyzed by flow cytometry.

B cells isolated from MG patients refractory to standard immunotherapy and with long (>4 years) disease duration (MG-Refractory) showed increased resistance to the treatment, with $CD27^{neg}$ compart being the most resistant. Nonetheless, we could not observe differences in B cell subsets composition between patients' cohorts before RTX treatment via flow cytometry. Therefore, we performed a deep immune phenotyping of B cells from the same patients' cohorts by mass cytometry employing a panel of 34 antibodies specific for surface antigens. This detailed analysis revealed naïve and double-negative B cells clusters specifically expanded in MG-Refractory patients compared to the other groups.

We then analyzed by scRNA-seq B cells isolated from an AChR-MG-Refractory patient with poor

response to RTX (P1), an AChR-MG patient with good treatment outcome (P2) and one HC. Sorted B cells were loaded in a Chromium iX platform and scRNA libraries were sequenced on a NovaSeq 6000 platform.

After dimensionality reduction with t-SNE and UMAP algorithm, B cells from HC and P2 clustered together while B cells from P1 formed a separate cluster. A differential expression analysis showed downregulation of MS4A1, encoding for the RTX target CD20, in the $CD27^{neg}$ compartment of B cells from P1, together with upregulation of several genes including CD83, CD69, FOS, JUN and HLA-DQB1. P1 upregulated genes analysis via Gene Ontology enrichment algorithm revealed enrichment in genes involved in positive regulation of lymphocyte activation. Moreover, gene network analysis performed using NetworkAnalyst software revealed that these genes insist on JNK, HRas and GSK3B pathways, respectively linked to B cell receptor engagement, autoantibodies production and survival of peripheral naïve B cells.

We believe our findings might significantly contribute to the identification of B cells expression profiles and fine subsets associated with treatment response, providing clinicians with valuable tools to personalize and optimize therapeutic strategies.

Keyword: *Myasthenia Gravis, B cells, autoantibody-mediated neurological disease, scRNA-seq, Mass cytometry*

#3 The emerging role of autoreactive T cells in Guillain-Barré syndrome

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Guillain-Barré syndrome (GBS) is a rare disabling disease of the peripheral nervous system (PNS). Although evidence from animal studies points to the involvement of pathogenic T lymphocytes targeting PNS-myelin antigens, the immune-mediated mechanisms in humans remain unknown. The aim of this study is to shed light on this issue by investigating the existence and providing an in-depth characterization of autoreactive T cells in GBS patients.

To this end we combined in vitro T cell screenings, generation of T cell clones, and high throughput TCRVb sequencing, revealing the presence of autoreactive memory $CD4^+$ and rare $CD8^+$ T cells targeting myelin antigens in the blood of GBS patients, but not in healthy individuals. The characterization of more than 900 autoreactive single $CD4^+$ T cell clones described a polyclonal TCR repertoire and short CDR3b lengths, a preferential HLA-DR restriction and immunodominant epitope recognition across patients. Autoreactive clonotypes were expanded in the blood of the same patient at different disease stages and, notably, were shared in the blood and cerebrospinal fluid of different GBS patients, but not in control individuals. Furthermore, autoreactive $CD4^+$ T cells were identified in the nerve biopsy of one GBS patient, thus further supporting their direct contribution to disease physiopathology.

Taken together, these findings provide the first description of autoreactive T cells in GBS patients, highlighting the potential existence of

public disease-associated autoreactive TCRVb clonotypes. This evidence solidifies the autoimmune aetiology of GBS and opens new perspectives in the broader field of inflammatory peripheral neuropathies with potential translational impact into biomedical applications.

Psychoneuroimmunology

#300 The presence of antibodies against muscarinic acetylcholine receptors in people with first episode psychosis predicts a worse clinical outcome at 12 months follow-up

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Pronounced reductions in muscarinic acetylcholine receptors (mAChR) in numerous brain regions, as assessed by RNA expression, protein levels, and radioligand binding assays, have been repeatedly observed in up to 25% of people with schizophrenia and other disorders associated with psychosis (1). We have also previously found elevated levels of antibodies targetting the m1AChR in people with established schizophrenia (2). Here, we have further investigated anti-mAChR antibodies in psychosis by collecting detailed clinical information and blood from 286 people experiencing a first episode of psychosis (FEP) and from 68 healthy individuals with no history of psychosis (as controls) and measuring the levels of antibodies against m1AChR and m2AChR in a live cell-based assay using mAChR-transfected CHO cells, to determine if the presence of these antibodies correlated with development of the FEP or any clinical features of disease. We then followed up patients 12 months later to determine if the presence of the antibodies against m1AChR and m2AChR at the time of the FEP showed associations with development of specific positive or negative symptoms of schizophrenia or any other feature of disease at follow-up.

The levels of antibodies against mAChRs were significantly elevated (>95th percentile of healthy controls) in 12.6% of people with a FEP (3 times more females than males) and correlated significantly (after correction for multiple comparisons) with several items on the 30-item Positive and Negative Symptom Scale (PANSS) at the FEP timepoint. The final diagnosis for the patients was determined at the 12-month timepoint, and patients were categorized into three diagnostic groups: persistent psychotic disorders (mostly schizophrenia), bipolar disorder, and brief psychotic disorders. Elevated levels of anti-M1 mAChR antibodies were seen more frequently in persistent psychotic disorders, but proportions of elevated levels of anti-M2 antibodies were similar in all subgroups. The presence of antibodies against either receptor at the onset of illness was associated with indicators of poorer functional outcomes after 12 months follow-up.

These studies suggest that immune dysfunction involving elevated levels of antibodies against mAChR occurs in a subgroup of people experiencing psychotic and mood disorders, not just schizophrenia, and that the presence of these antibodies correlates with worse functional outcomes for patients.

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Keyword: *psychosis, schizophrenia, autoantibodies*

#142 Interaction between a high fat diet and stress: effect on depression in relation to the gut microbiome-endocannabinoidome axis

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Objectives/Background: Depression is a widespread neuropsychiatric disorder that can affect any population, regardless of age and socioeconomic condition. Several studies have linked the pathophysiology and regulation of mood disorders, particularly depression, with the modulation of the endocannabinoid system and endocannabinoidome (eCBome). The eCBome is involved in the regulation of the gut microbiota while the composition of the gut microbiota influences the activity of the eCBome. In recent years, it has been shown that a high-fat diet (HFD) is associated with an increase in the tone of the eCBome, concomitant with a dysregulation of the gut microbiota. In addition, HFD can lead to depression, although the causal mechanisms are not yet clear. In our study, we aimed to determine the interactions between HFD and depression caused by chronic stress in the context of the gut-brain axis.

Method: We conducted a 9-week standardized chronic unpredictable mild stress (UCMS) model to induce depression in two groups of mice fed either a low-fat, low-sucrose diet (LFLS) or a high-fat, high-sucrose diet (HFHS). Throughout the study, fecal samples were collected, and body composition analyses were performed at week 0, week 5, and week 9 to assess the composition of the microbiota (16S gene amplicon sequencing) and to monitor changes in total lean mass and fat mass over time. At week 9, behavioral tests were performed to assess depressive and anxiety-like symptoms. Gene expression analysis was performed to assess changes in blood-brain barrier (BBB) integrity, inflammation, and neurotransmitter levels in the brain and gut. In addition, short-chain fatty acids were analyzed.

Preliminary results: We confirmed the presence of depressive-like signs in the stressed group, highlighting that an HFHS diet exacerbated depressive-like symptoms. Using multivariate analyses, we found that stress had a major impact on the microbiota change compared to diet. For example, Bifidobacterium increases with the HFHS diet and decreases with stress, suggesting that this taxon is associated with the development of depression, while Oscillobacter decreases only in the no-stress group, suggesting that this bacteria is a key player of the development of the higher depressive-like

symptoms found in HFHS stress group. Additionally, butyric acid is decreased in the HFHS stress group compared to the LFLS stress group, indicating a potential role in the development of depression.

Perspectives: The measurement of neuroinflammation and neurotransmitters is ongoing, and the results will be shared during the presentation. Multi-correlations with the gut microbiota, short-chain fatty acids, neurotransmitters, and behavior will be explored using Mixomics analysis.

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Keyword: *depression, high-fat diet, microbiome, neuroinflammation, gut-brain axis*

#12 Behavioral as well as hippocampal transcriptomic and microglial responses differ across sexes in adult mouse offspring exposed to a dual genetic and environmental challenge

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Introduction: A wide range of positive, negative, and cognitive symptoms compose the clinical presentation of schizophrenia. Schizophrenia is a multifactorial disorder in which genetic and environmental risk factors interact for a full

emergence of the disorder. Infectious challenges during pregnancy are a well-known environmental risk factor for schizophrenia. Also, genetic variants affecting the function of fractalkine signaling between neurons and microglia were linked to schizophrenia. Translational animal models recapitulating these complex gene-environment associations have a great potential to untangle schizophrenia neurobiology and propose new therapeutic strategies. **Methods:** Considering that genetic variants affecting the function of fractalkine signaling between neurons and microglia were linked to schizophrenia, we compared the outcomes of a well-characterized model of maternal immune activation using the viral mimetic polyinosinic:polycytidylic acid (Poly I:C) in wild-type versus fractalkine receptor knockout mice. Possible behavioral and immune alterations were assessed in male and female offspring at adulthood. Considering the role of the hippocampus in schizophrenia, microglial analyses and bulk RNA sequencing were performed within this region to assess the neuroimmune dynamics at play. **Results:** Offspring exposed to the dual challenge paradigm exhibited symptoms relevant to schizophrenia and unpredictably mood disorders, which differed between males and females. Males displayed social and cognitive deficits related to schizophrenia, while females mainly presented anxiety-like behaviors related to mood disorders. Hippocampal microglia in females exposed to the dual challenge were hypertrophic, indicative of an increased surveillance, whereas those in males showed on the other end of the spectrum blunted morphologies with a reduced phagocytosis. Hippocampal bulk-RNA sequencing further revealed a downregulation in females of genes related to GABAergic transmission, which represents one of the main proposed causes of mood disorders. **Conclusions:** Building on previous results, we identified in the current study specific behavioral phenotypes in female mice exposed to a dual genetic and environmental challenge, thus suggesting a new model of neurodevelopmentally-associated mood and affective symptoms. This opens the field to new investigations into the susceptibility to stress using a model based on genetic and immune vulnerability as presented here.

Keyword: *Schizophrenia, mood disorders, microglia, maternal immune activation, GABA*

#172 Psychological stress exacerbates immunoglobulin E-dependent chronic allergic skin inflammation via suppression of M2 macrophage-induced efferocytosis

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Psychological stress is known to exacerbate allergic diseases, including atopic dermatitis; however, the mechanisms underlying this effect remain unclear. In this study, we investigated the molecular and cellular mechanisms associated with chronic restraint stress (CRS)-induced exacerbation of immunoglobulin (Ig)E-dependent chronic allergic skin inflammation (IgE-CAI) in mice. CRS increased ear thickness and eosinophil infiltration in lesions. Chemical sympathetic denervation with 6-hydroxydopamine abolished the exacerbation, whereas adrenalectomy had minimal effect. Exogenous norepinephrine (NE) or β2-adrenergic receptor (Adrb2) agonist administration promoted the exacerbation, and

Adrb2 deficiency suppressed it, suggesting that NE released from sympathetic nerves is involved in CSR-induced IgE-CAI exacerbation via Adrb2 activity. Among the infiltrating leukocytes in IgE-CAI skin lesions, M2 macrophages (M2-Macs) expressed high levels of Adrb2, and we hypothesized that CRS affects the anti-inflammatory function of M2-Macs, which precipitates IgE-CAI exacerbation. Adoptive transfer of CD115+ monocytes from CRS mice resulted in only mild reversal of IgE-CAI exacerbation, indicating that M2-Macs derived from inflammatory monocytes subjected to CRS have diminished anti-inflammatory activity against IgE-CAI. Further analysis of molecules within M2-Macs that contribute to CRS-induced IgE-CAI exacerbation revealed that M2-Macs showed significantly lower expression of efferocytosis-related genes such as Gas6, MerTK, and CD163 in lesions in mice subjected to CRS. Efferocytosis refers to the process of engulfing apoptotic cells to prevent their secondary necrosis and release of damage-associated molecular patterns that promote inflammation. We investigated the efferocytic capacity of M2-Macs derived from mice subjected to CRS and observed that these mice had significantly lower efferocytic capacity of M2-Macs and more dead cells in the lesions. In conclusion, our results suggest that psychological stress suppresses the efferocytic activity of M2-Macs via Adrb2, which may serve as a novel therapeutic target for allergic diseases.

Keyword: *psychological stress, Atopic dermatitis, M2-macrophage, efferocytosis, sympathetic nerve*

#400 Neurovascular mitochondrial susceptibility impacts blood-brain barrier function and behavior

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The blood-brain barrier (BBB) is critical to optimal brain function, and its impairment has been linked to multiple neurological disorders. A notable feature of the BBB is its elevated mitochondrial content compared to peripheral endothelial cells, although the functional implications of this phenomenon remain unknown. Here we studied BBB mitochondrial function in the context of the 22q11.2 deletion syndrome (22qDS), a condition associated with a highly increased risk for neuropsychiatric disease. As the 22q11.2 deletion includes 6 mitochondrial genes, and because we have previously identified BBB impairment in 22qDS, we addressed the hypothesis that mitochondrial deficits contribute to BBB dysfunction and impact behavior in this condition. We report mitochondrial impairment in human induced pluripotent stem cell (iPSC)-derived BBB endothelial cells from 22qDS patients, and in BBB endothelial cells from a mouse model of 22qDS. Remarkably, treatment to improve

mitochondrial function attenuates mitochondrial deficits and enhances BBB function in both the iPSC and mouse 22qDS models. This treatment also corrected social memory in 22qDS mice, a deficit previously associated with BBB dysfunction. As we find that BBB integrity is correlated with social memory performance, together our findings suggest that mitochondrial dysfunction in the BBB influences barrier integrity and behavior in 22qDS.

August 22

Involvement of glial cells in neuroinflammation

#116 Interleukin-3 coordinates glial-peripheral immune crosstalk to incite multiple sclerosis

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Glial cells and central nervous system (CNS)-infiltrating leukocytes contribute to multiple sclerosis (MS). However, the networks that govern crosstalk among these ontologically distinct populations remain unclear. Here, we show that, in mice and humans, CNS-resident astrocytes and infiltrating CD44hiCD4+ T cells generated interleukin-3 (IL-3), while microglia and recruited monocytes, macrophages, and dendritic cells, responded to the cytokine by expressing interleukin-3 receptor-α (IL- 3Rα). Astrocytic and T cell IL-3 elicited immune migratory, recruitment, and chemotactic programs by IL-3Rα+ myeloid cells that enhanced CNS immune cell infiltration, neuroinflammation, and demyelination; exacerbating MS and its preclinical model. Multiregional single-nuclei RNA sequencing (snRNAseq) of human CNS tissue

from unaffected controls and MS patients reveals the appearance of a subset of IL3RA-expressing myeloid cells in MS plaques with unique recruitment, migratory, and chemotactic programing and function. In humans with MS, IL3RA expression by plaque myeloid cells and IL-3 levels in the cerebrospinal fluid (CSF) predict myeloid and T cell abundance and recruitment into the CNS. Together, our findings establish IL-3:IL-3RA signaling as a bidirectional glial-peripheral immune crosstalk network that promotes neuroinflammation, prompts immune cell recruitment to the CNS, and worsens MS.

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Keyword: *Interleukin-3, Multiple sclerosis, microglia, astrocytes*

#9 Fibulin-2 impedes oligodendrogenesis through engaging the Notch signaling pathway

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Remyelination is a neuroprotective repair response to demyelination that decreases the vulnerability of axons to irreversible degeneration. It is a highly efficient process that occurs extensively at early stages of multiple sclerosis (MS), but it eventually fails with disease progression. A contributor to inefficient remyelination is the altered extracellular matrix (ECM) in lesions that prevents recruitment and/or differentiation of oligodendrocyte precursor cells (OPCs). Using bioinformatic analysis of published proteomic and RNA databases for MS and its experimental autoimmune encephalomyelitis (EAE) model, we have identified fibulin-2 (FBLN2) as a highly upregulated ECM member in lesions. Here, we examined the expression of FBLN2 in lesions of MS and EAE, and addressed whether this ECM molecule could affect oligodendrocyte maturation and remyelination.

By immunofluorescence confocal microscopy and Imaris 3D rendering, we found increased levels of FBLN2 in EAE and MS lesions, localized to $GFAP^+$ astrocytes. Here, we studied if in vivo astrocytic deletion of FBLN2 using adeno-associated viruses (AAV)- CRISPR/Cas9 system impacts EAE disease course and severity. FBLN2 deficiency in astrocyte resulted in a better clinical recovery during EAE which was correspondent with more mature oligodendrocytes. Moreover, FBLN2 knockout mice showed more robust oligodendrogenesis following lysolecithin (LPC)-induced demyelination, indicating the inhibitory effect of FBLN2 on oligodendrocyte. Single cell RNA sequencing identified myelinating OL as the most abundant population of oligodendrocyte lineage cells in FBLN2 deficient mice post-peak EAE. These findings are in accordance with cell culture experiments where FBLN2 impaired maturation of human or mouse OPCs to oligodendrocytes and induced cell death. Blocking Notch signaling pathway by SiRNA or pharmacological approaches overcame the FBLN2 inhibition, highlighting the role of Notch

signaling pathway in FBLN2-induced impaired oligodendrogenesis.

Overall, these results suggest FBLN2 as a new extracellular matrix inhibitor of oligodendrocytes and myelin repair through engaging the Notch signaling pathway. Overcoming FBLN2 has the therapeutic potential to improve remyelination and prognosis in MS.

Influence of sex on neuroinflammation

#305 Role of sex, gonadal hormones and dutasteride treatment on central and peripheral inflammation in a mouse model of Parkinson's disease.

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Prevalence and incidence of Parkinson's disease (PD) are higher in men than women suggesting a possible role of gonadal hormones (GH) in protection from neurodegeneration and neuroinflammation. Dutasteride (DUT), a 5alpha-reductase (5aR) inhibitor already in clinical use by men, modulates endogenous levels of GH through inhibition of the 5aR enzyme. Our hypothesis was that DUT protects from neurodegeneration and neuroinflammation in a mouse model of PD.

Because PD causes neurodegeneration and neuro-inflammation in the central nervous system (CNS) and in the enteric nervous system (ENS), our objective was therefore to study the impact of DUT, GH and biological sex in PD in both nervous systems.

Four groups of mice, male or female, were gonadectomized (GDX, to model andropause and menopause) or sham-operated (SHAM). On the 5th day they received DUT or vehicle for 10 days and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to model PD, or saline.

MPTP caused dopamine loss and an astrocyte activation in all groups except in SHAM females and in SHAM males treated by DUT.

MPTP caused an increase in microglial density in male that was prevented by DUT in SHAM males. Females did not manifest MPTP-induced microglial density increase.

We observed, for the first time in a mouse model of PD, the presence of doublets of microglial cells (Figure 1). They result from microglial proliferation in response to inflammation. This microglial proliferation was present in MPTP-treated male groups but not in female. In SHAM males, DUT had a protective effect.
Three-dimensional morphological analysis of microglia (Figure 2) showed an activation caused by MPTP in males but not in females. This microglial activation was characterized by the shift from quiescent to activated morphology with smaller and less ramified microglial cells. DUT was not protective against MPTP-induce microglial activation.

We are currently analyzing the ENS from the myenteric plexus of these mice to assess dopaminergic neuron loss and macrophages proliferation and compare results between CNS and ENS.
We show that GH modulation properties of DUT, GH and/or biological sex can exert protection in a mouse model of PD.

Figure 1:

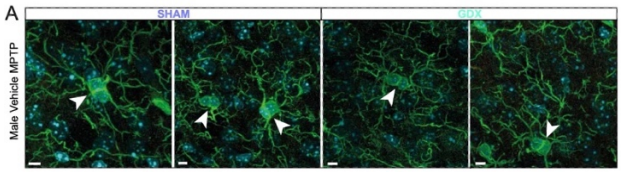
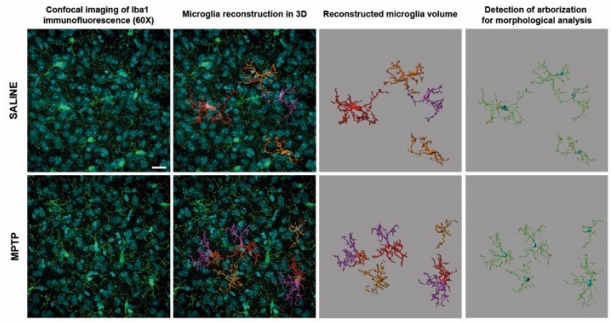


Figure 2:



Keyword: *Parkinson's disease, Sex differences, Microglial activation, Microglial morphology, Dutasteride*

Microglia in neural development, remodelling, and protection

#289 Avb8-Tgfb1 signaling in brain vascular and microglial development

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The early formation of the brain involves multiple cellular and molecular interactions which coordinate both vascular and immune cell differentiation in concert with the production of neurons, oligodendrocytes and astrocytes. Here, we show that the development of both neuroimmune and neurovascular systems depend on integrin beta 8-dependent activation of latent TGFb by embryonic brain stem cells. Domain-restricted deletion of Itgb8 in these progenitors establishes complementary domains of developmentally arrested "dysmature" microglia and homeostatic microglia that persist into adulthood. In the absence of autocrine Tgfb1 signaling, microglia adopt a similar reactive microglial phenotype, leading to astrogliosis and neuromotor symptoms almost identical to Itgb8 mutant mice. By comparing mice with genetic deletions in critical components downstream of Itgb8, we show that non-canonical (Smad-independent) signaling acts to partially suppress the dysmature microglia phenotype and associated neuromotor dysfunction. Finally, we show that dysmature microglia share properties of disease-associated microglia, providing evidence for a connection between the signaling pathways that regulate microglial homeostasis in development and in the context of injury or disease.

#128 Stearoyl-CoA desaturase-1 impairs the regenerative and anti-inflammatory properties of microglia and T cells in the brain

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The imbalance between pathogenic and protective immune cell subsets is a cardinal feature of neuroinflammatory disorders. Emerging evidence indicates that endogenous changes in fatty acid metabolism have a major impact on microglia and T cell physiology in neurological disorders. To date, however, the molecular mechanisms that underpin the impact of fatty acid metabolism on immune cell physiology and brain pathology in these disorders remain poorly understood. Here, we report that stearoyl-CoA desaturase-1 (SCD1), an enzyme essential for the desaturation of fatty acids, acts as an endogenous brake on brain repair and augments autoimmune-mediated demyelination. Genetic deficiency and pharmacological inhibition of SCD1 enhanced remyelination and attenuated neuroinflammation by promoting the formation of reparative microglia and regulatory T cells (Tregs), respectively. Guided by RNA sequencing and lipidomics analysis, we find that absence of SCD1 improves the metabolic phenotype of both microglia and T cells. Specifically, absence of SCD1 enhanced the intracellular processing of myelin-derived lipids by microglia, thereby preventing lipotoxicity and inducing a disease-resolving microglia phenotype. Mirroring these findings, loss of SCD1 in T cells increased the hydrolysis of anti-inflammatory polyunsaturated fatty acids that induced Treg differentiation through activating the peroxisome proliferator-activated receptor gamma receptor. In aggregate, our findings identify SCD1 as an essential determinant of immune cell function, neuroinflammation, and brain repair, with potentially broad implications for the development of novel therapeutic strategies for neuroinflammatory disorders.

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Keyword: *Fatty acid metabolism, Neuroinflammation, remyelination, Regulatory T cells, Macrophages*

#17 APOE4 Impairs Microglia Response to Neurodegeneration in Alzheimer's Disease

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** These authors equally contributed to this study.*

Objectives

APOE4 is the strongest genetic risk factor for late-onset Alzheimer's disease (AD). We previously identified that APOE signaling governs the transcriptional regulation from homeostatic to neurodegenerative microglia (MGnD). However, the underlying mechanism for APOE4-mediated microglial dysregulation and its contribution to increased risk for developing AD, is unknown.

Methods

Here we aimed to dissect the impact of microglial APOE4 on AD pathology, using CX3CR1-CRE^{ERT2} mice crossed to APOE-KI(E3 and E4)$^{fl/fl}$:APP/PS1.

Results

We identified reduced numbers of Clec7a^{+} MGnD-microglia per plaque in APP/PS1:APOE4-KI mice, despite their increased plaque load, compared with APP/PS1:APOE3-KI mice. Moreover, APOE4-KI mice challenged with labeled apoptotic neurons, failed to respond to acute neurodegeneration, depicted by reduced numbers of phagocytic MGnD-microglia at injection site compared with APOE3-KI mice. Here we show that conditional genetic deletion of APOE4 in microglia resulted in increased numbers of MGnD-microglia in response to acute neurodegeneration and in APP/PS1 mice. scRNAseq analysis showed increased proportion of MGnD-microglia in APP/PS1:APOE4 conditional KO mice, associated with reduced plaque pathology and increased astrocytic recruitment towards plaques. Furthermore, we show impaired induction of MGnD signature in AD brains of APOE4 carriers.

Conclusions

Our findings show that APOE4 is a negative regulator of MGnD-microglia in AD, and that its genetic deletion restores the induction of MGnD signature associated with reduction in plaque pathology. Taken together, these findings identify a cell-intrinsic role of APOE4 in the induction of dysfunctional MGnD microglia and their impaired response to neurodegeneration, which may provide new molecular targets to modulate and restore functional microglia in AD.

#83 Microglia coordinate cellular interactions during spinal cord repair in mice

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Traumatic spinal cord injury (SCI) triggers a neuro-inflammatory response dominated by tissue-resident microglia and monocyte derived macrophages (MDMs). Since activated microglia and MDMs are morphologically identical and

express similar phenotypic markers in vivo, identifying injury responses specifically coordinated by microglia has historically been challenging. Here, we pharmacologically depleted microglia and use anatomical, histopathological, tract tracing, bulk and single cell RNA sequencing to reveal the cellular and molecular responses to SCI controlled by microglia. We show that microglia are vital for SCI recovery and coordinate injury responses in CNS-resident glia and infiltrating leukocytes. Depleting microglia exacerbates tissue damage and worsens functional recovery. Conversely, restoring select microglia-dependent signaling axes, identified through sequencing data, in microglia depleted mice prevents secondary damage and promotes recovery. Additional bioinformatics analyses reveal that optimal repair after SCI might be achieved by co-opting key ligand-receptor interactions between microglia, astrocytes and MDMs.

Keyword: *microglia, macrophage, astrocyte, neurodegeneration, demyelination*

T cells in Neurological diseases

#11 TCF1 is a determinant of homeostatic versus pathogenic Th17 cell state

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CD4$^+$ T helper 17 (Th17) cells encompass a spectrum of cell states including homeostatic cells that maintain physiological functions such as barrier integrity and pathogenic cells that drive autoimmune tissue inflammation. Identifying the regulators that determine these cell states will provide means to control tissue inflammation without compromising the physiological functions of Th17 cells. Here, we identified TCF1 as the key regulator that determines Th17 cell state. IL-23, a cytokine critical for inducing pathogenic Th17 cells, decreased TCF1 expression. Consistent with this observation, conditional deletion of Tcf7/TCF1 in mature myelin-specific T cells conferred pathogenicity to homeostatic Th17 cells independent of IL-23. Conversely, sustained TCF1 expression impaired acquisition of pathogenicity. Integration of transcriptional and chromatin accessibility data showed that TCF1 maintained homeostatic state through a regulatory network involving ETS family transcription factors, EGR1, and FOXO1 and by binding to and interfering with RORgt activity. Our findings provide mechanistic insight into how the homeostatic and pathogenic Th17 cell states are determined and into the association of genetic variants in TCF7 with susceptibility to central nervous system (CNS) autoimmunity.

#193 Tissue-resident memory T cells sustain the chronic phase of experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is a chronic inflammatory disease characterized by demyelinating lesions of the central nervous system (CNS) likely due to uncontrolled activation of auto-reactive T lymphocytes. Therapeutic options against MS include the use of molecules preventing T cell migration to the CNS. However, while these molecules are showing significant clinical effect, a large proportion of patients still experience disease relapse and for a proportion of them a secondary progressive disease course. We and others recently reported that the development of autoreactive brain resident memory T cells (Trm) could be an important mechanism sustaining the chronic course of CNS autoimmune pathologies in mouse models of CNS inflammatory diseases. Moreover, in human, recent works showed the presence of Trm in the brain lesions of MS patients. We postulated here that brain Trm could actively promote a compartmentalized inflammation behind the blood brain barrier contributing to disease progression and lack of CNS regeneration contributing to the secondary progression phase in MS patients.

Using the model of active experimental autoimmune encephalomyelitis (EAE) in mice, our results showed that bona fide autoreactive $CD4^+$ Trm cells effectively developed within the brain and spinal cord during the chronic phase of EAE. These cells expressed the Trm-specific transcription factor Hobit as well as classical Trm markers (CD69, Cxcr6, P2x7r, CD49a) and were insensitive to the MS treatment blocking agent FTY720. Moreover, CNS-infiltrating Trm displayed a proinflammatory profile with high expression of IL-17, IFNg and TNF, and in accordance with their proinflammatory phenotype, were preferentially localized within EAE inflammatory lesions. Our single-cell transcriptomic analysis revealed phenotypic and functional heterogeneity of CNS-infiltrating Trm $CD4^+$ T cells, suggesting distinct contributions of specific Trm cell subsets to the disease process. Finally, while antibody-mediated depletion of peripheral $CD4^+$ T cells had no impact on the disease severity, pharmacologic or genetic targeting of the Trm compartment reduced disease severity during the chronic phase. These data demonstrate for the first time that the chronic phase of EAE is actively maintained by a local inflammation sustained by proinflammatory Trm cells. Our results suggest that new therapeutic strategies for the treatment of MS should consider targeting not only peripheral recirculating T cells but also the CNS-resident T cell compartment.

Keyword: *EAE, Trm, Chronic inflammation*

#16 Brain endothelial antigen presentation detains CD8 T cells at the blood-brain barrier and contributes to its breakdown

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Blood-brain barrier (BBB) breakdown and immune cell infiltration into the central nervous

system (CNS) are early hallmarks of multiple sclerosis (MS). High numbers of CD8 T cells are found in MS lesions and antigen (Ag)-presentation at the BBB was proposed to promote CD8 T-cell entry into the CNS. Employing live cell imaging and an in vitro model of the BBB and the ODC-OVA mouse as in vivo model of CD8 T-cell mediated CNS autoimmunity we show that brain endothelial cells process and cross-present antigens leading to effector CD8 T-cell differentiation. Under physiological flow, endothelial Ag-presentation prohibited CD8 T-cell crawling and diapedesis leading to brain endothelial apoptosis. Brain endothelial Ag-presentation in vivo was limited due to efficient Ag uptake by professional CNS resident macrophages but still reduced motility of Ag-specific CD8 T cells within CNS microvessels. Thus, neuroinflammatory conditions leading to luminal MHC class I restricted Ag- presentation at the BBB will slow or prohibit CD8 T-cell entry into the CNS and rather trigger CD8 T-cell mediated focal BBB breakdown.

Keyword: *Blood-Brain Barrier, CD8 T cell, Multiple Sclerosis, Imaging*

#355 Impaired CNS Immunosurveillance by CCR7+ CD4 T cells during chronic neuroinflammation

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CD4 T cells critically regulate immune homeostasis in the brain, yet their functions in this context remain poorly understood. Understanding the adaptive immune mechanisms operating under normal, baseline conditions is paramount to effectively develop interventions that can mitigate potential dysfunctions observed during neurodegenerative disorders. Utilizing a combination of cutting-edge methodologies such as single-cell (sc) transcriptomic analysis, Assay for Transposase-Accessible Chromatin (ATAC)-seq, spatial transcriptomics of the hippocampus, and flow cytometry, we conducted an in-depth investigation of the non-inflamed rhesus brain. In this Abstract, we present an intriguing finding regarding the existence of CCR7+ CD4 T cells within the brain parenchyma. Notably, these cells exhibited molecular signatures that closely resembled those of bona fide central memory CD4 T cells (T_{CM}) found in the spleen (**see Figure 1**). Moreover, our ATAC-seq analysis provided further insights into the epigenetic profiles of these CCR7+ CD4 T cells, revealing striking similarities to T_{CM}. Specifically, we observed pronounced chromatin accessibility at the CCR7, CD28, and Bcl-6 loci, defining molecular features of T_{CM}. By employing high-resolution flow cytometry, we successfully distinguished phenotypic differences between CD28+ CCR7+ cells, present in the brain parenchyma, and CD28-CD69+ cells, putative resident memory cells within the brain. Furthermore, our findings demonstrate the functional competence of brain CCR7+ CD4 T cells, as evidenced by their ability to undergo recall proliferation and produce interleukin 2 (**see Figure 2**). Spatial profiling of the hippocampus revealed that these T cells predominantly reside in perivascular and parenchymal regions, with the skull bone marrow

emerging as a potential local niche for CCR7+ CD4 T cells. Importantly, we discovered that sequestering T_{CM} cells in lymph nodes using the sphingosine 1 receptor antagonist resulted in reduced frequencies of CCR7+ CD4 T cells in the cerebrospinal fluid, accompanied by immune activation in this compartment. Intriguingly, our study revealed a remarkable depletion of CCR7+ CD4 T cell frequencies in the brains of animals with chronic SIV infection. This depletion coincided with neuroinflammation, as evidenced by microglial activation and the upregulation of gene programs associated with neurodegeneration (eg., CD44, FNDC3B, RALGAP2, RGCC), in myeloid cells. This observation suggests a potential link between the absence of CCR7+ CD4 T cells and the development of neuroinflammation in the context of chronic SIV infection (**see Figure 3).** Altogether, our findings underscore the critical role of CCR7+ CD4 T cells in immune surveillance within the CNS and highlight their potential as valuable therapeutic targets to mitigate the development or progression of neurodegenerative diseases.

Figure 1: Single-cell transcriptomic analysis reveals previously uncharacterized CD4 and CD8 T_{CM} subsets in brain.

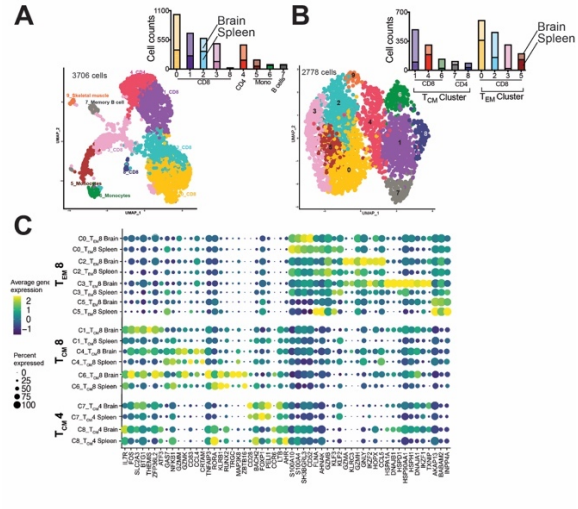


Figure 2: CCR7+ CD4 T cells in the brain mount recall responses and produce IL-2 ex vivo.

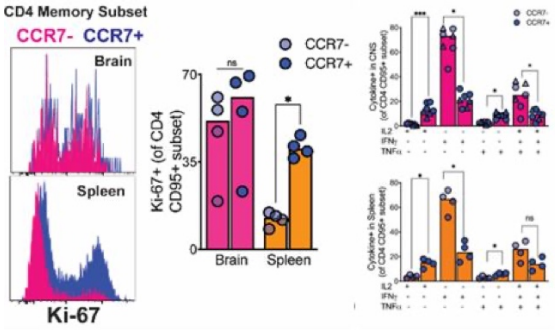
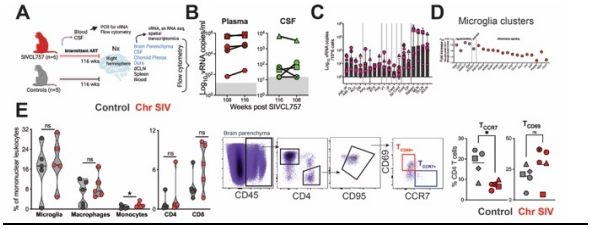


Figure 3: Preferential depletion of parenchymal CCR7+ CD4 T cells in chronic SIV infection.



#102 The transcription factor c-Maf promotes immunoregulation of CD8+ T cells in multiple sclerosis

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Objectives

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. CD8+ T cells are prominent in inflammatory lesions. Recent advances in the understanding of immune checkpoint molecules, including programmed cell death-1 (PD-1) expressed on CD8+ T cells, highlight the immunoregulatory role of this T cell subset; however, the role of CD8+ T cells in MS is unclear.

Therefore, we aimed to characterize PD-1-expressing (PD-1+) CD8+ T cells in MS.

Methods
We analyzed the immune profiles of T cells from both the cerebrospinal fluid (CSF) and peripheral blood of 45 patients with MS or clinically isolated syndrome (CIS) and peripheral blood of 12 healthy individuals. We further analyzed the transcriptome of sorted PD-1+CD8+ T cells obtained from interferon-beta treated patients and validated their regulatory machinery using in vitro cell culture assays with lentiviral gene transfer.

Results
The proportion of memory cells in CD4+ T cells or CD8+ T cells was higher in the CSF from patients with MS or CIS during a disease flare than in the peripheral blood from healthy subjects or patients with MS. During the disease flare, we found that PD-1+CD8+ memory T cells were enriched in the cerebrospinal fluid, which predicted a better outcome after intravenous steroid therapy. In the remission state, PD-1+CD8+ T cells were decreased in the peripheral blood of patients with MS and resolved in patients treated with interferon-beta. Transcriptome analysis of sorted PD-1+CD8+ T cells identified the transcription factor c-Maf as a key regulator of the gene module, including several co-inhibitory molecules. Furthermore, c-Maf expressed in CD8+ T cells induced PD-1 expression and interleukin 10 (IL-10) production and suppressed the survival of alloactivated CD4+ T cells.

Conclusion
This study uncovered a beneficial role of PD-1+CD8+ memory T cells against MS and demonstrated that c-Maf-driven IL-10 is an immunoregulatory machinery.

Keyword: *Multiple Sclerosis, CD8+ T cells, c-Maf, PD-1*

Biomarkers of neuroinflammation

#59 CanProCo Study; Examining Prognostic Protein Biomarkers in Sera and Plasma from Multiple Sclerosis Patients

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Background: The heterogenic clinical presentation and unpredictable disease trajectory of patients with multiple sclerosis (MS) remains a prevalent clinical challenge. Prognostic biomarkers are much needed but are lacking. The Canadian Prospective Cohort to Understand Progression in MS (CanProCo) study involves collection of biological, radiological, and clinical data from 1000 MS patients during early disease over five years. **Objective**: Investigate protein biomarkers in sera and plasma from MS patients. **Method:** Unbiased clinical clusters were determined from detailed clinical data from 933 CanProCo patients using PHATE embedding. 71 immune-related proteins were quantified by nELISA (Eve Technologies) in matched plasma and serum samples from 125 MS patients. Detailed clinical parameters at collection date and one-year follow-up were examined with supervised approaches to stratify patients into clinical clusters. Protein levels were then compared across clinical clusters. The delta EDSS was calculated following one-year follow-up to

examine the prognostic value of each protein. **Results:** There was a disparity in the protein composition between serum and plasma in MS patients. Eight distinct clinical clusters were identified. Eotaxin (CCL11) levels in serum and plasma were elevated in a clinical cluster with mostly PPMS patients with high EDSS, and slightly lower in clusters that defined RRMS patients. CXCL9 and CXCL10 in serum were correlated with MS disease worsening within one year, as defined by EDSS. Matched sera and plasma when analyzed together improved the prognostic power of this association. **Conclusion:** Collation of clinical and radiological data spanning five years will be eventually analysed to examine the predictive power of the soluble proteins over the course MS disease. These data present promising results of the use of novel and pragmatic protein biomarkers to predict MS disease progression.

Keyword: *Biomarker, Protein Biomarkers, Multiple Sclerosis, Serum, Plasma*

#286 Machine learning identifies a combination of immunological and clinical parameters as the best predictor of MS disease progression

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Background: Multiple Sclerosis (MS) is thought to be initiated by pathogenic T effector cells (Teff, $CD4^+$ and $CD8^+$) migrating into the central nervous system (CNS). Regulatory T cells (Treg) are a key component of immune tolerance and protect against autoimmune disease. Teff and Treg use cellular adhesion molecules (CAMs) and chemokine receptors (CCR) to migrate towards and into the CNS. Currently, there are no good predictors of MS disease progression. This study sought to investigate whether the CAM and CCR signature on Teff and Treg, combined with clinical information, can predict MS progression.

Objectives & Aims: Unravel the mechanisms by which Teff and Treg cross the BBB into CNS and investigate whether this migratory signature in combination with clinical data can predict disease progression.

Methods: The expression of cellular adhesion molecules LFA-1, VLA-4, ALCAM, CD6, MCAM, P-selectin, L-selectin and αvβ3 has been studied on Teff and Treg present in the peripheral blood of MS patients (N=141; RRMS, CIS, SPMS, PPMS) and healthy controls (N=43; HC) using FACS. Furthermore, the expression of chemokine receptors CCR2, CCR3, CCR5, CCR6, CXCR4 and CX3CR1 and Treg functional markers ST2L, PSGL-1, CD147 have also been investigated. Subsequently these data have been correlated with patient data (retrospective and prospective clinical parameters reflective of MS disease course e.g., EDSS, MRI lesion load, annual relapse

rate, 3-5 year follow up). Using classical, supervised machine learning (ML) (ensemble methods), as well as semi-supervised learning (PHATE, MELD), we investigated which immune, clinical, and MRI parameters contribute to disease progression.

Results: VLA-4, MCAM, CD6, ST2L, PSGL-1, CCR3, CCR6, and CXCR4 are differentially regulated in MS patients compared to healthy control individuals. Treg expression of CCR5 and CCR6 seems to be linked to progressive MS disease types. We identified two clinical (sex and presence of pyramidal deficits according to EDSS subscale) and two immune parameters ($CD8^+CD6^+$ and $TregST2L^+$ cell populations) as strong predictors of disease progression. The robustness of the prediction algorithm was improved when clinical and immune models were combined.

Conclusions: The migratory signature of Treg and Teff is different in MS patients compared to healthy individuals, and within MS subtypes, which may have an effect on disease progression. The preliminary ML findings support our hypotheses that a combination of clinical and immunological data builds the best prediction model for disease progression.

Keyword: *Multiple Sclerosis, Machine Learning, Cellular Adhesion Molecule, Chemokine Receptor, T cells*

#266 Biomarkers in autoimmune diseases of the central nervous system : the autoimmune encephalitis challenge

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Autoimmune encephalitis (AIE) refers to a group of rare diseases characterized by a dysimmune-induced encephalitic impairment, which could be a primary autoimmune, postinfectious, paraneoplastic, or immunotherapy-induced disorder. Despite constant improvement in autoantibodies identification allowing better diagnosis, treatment remains empirical. AIE evolution is extremely variable, raising questions regarding the immunotherapy's management, duration and intensity. Thus, one of the main issues is to differentiate active neuroinflammation from sequelae, and consequently to guide therapeutic decisions. To date, most clinical manifestations and paraclinical tests (Magnetic resonance imaging, CSF usual markers, autoantibodies levels) used to diagnose AIE have proven to be unreliable for use in the follow-up.
Nine proteins were tested in both serum and cerebrospinal fluid (CSF) at diagnosis (T0) and during the follow-up (T1) in patients with autoimmune encephalitis and correlated with clinical status in order to assess their value as neuroinflammation biomarkers.

We found YKL-40, a cytokine-like proinflammatory protein produced by glial cells, is correlated in the CSF with the clinical course of AIE in our cohort. YKL-40 appeared to be a viable and interesting biomarker. A prospective study is now mandatory to confirm this potential biomarker of neuroinflammatory activity. A systematic and regular analysis of YKL-40 could also assess its ability to predict relapses.

#310 Interleukin-7 receptor alpha is increased on CD4+ effector T cells in clinically established multiple sclerosis

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The radiologically isolated syndrome (RIS) describes individuals without typical multiple sclerosis (MS) symptoms but with characteristic magnetic resonance imaging (MRI) lesions in the brain seen in people with multiple sclerosis (pwMS). (1) Within ten years of diagnosis, approximately half of all people with RIS (pwRIS) are diagnosed with MS. (2) Previous research has shown that younger age (<37 years), positive cerebrospinal fluid-specific oligoclonal bands, spinal cord or infratentorial lesions, and developing new gadolinium-enhancing lesions on follow-up MRI are independent predictors of RIS developing MS. (2,3) While people with RIS (pwRIS) are at high risk of developing MS, the mechanisms underlying the conversion from RIS to MS are incompletely understood. We hypothesize that studying the immune mechanisms underlying RIS will provide a better understanding of the disease processes leading to MS and help identify novel prognostic biomarkers for pwRIS. We measured the immune cell activation profiles in the peripheral blood of pwRIS, pwMS and healthy controls using mass cytometry (cytometry by time of flight, or CyTOF). We used a 37-marker panel to characterize T cell subsets in our cohort of 16 healthy controls, 24 pwRIS, and 18 pwMS. We observed a numerical but non-significant increase of interleukin-7 receptor alpha (IL7R-alpha) in pwRIS and a statistically significant increase of IL7R-alpha in pwMS on effector T cell populations, including CD4+ terminal effector T cells and CD4+ T helper 17 cells ($p<0.05$). Previous studies have shown that common variants at the IL7R-alpha, encoding its receptor, are associated with susceptibility to MS. (4) Our data suggest that increased IL7R-alpha expression on effector T cells occurs in clinically established MS and, to a lesser degree, in RIS. Whether a subgroup of pwRIS with high IL7R-alpha expression on effector T cells is at increased risk of developing symptoms and receiving a diagnosis of MS will be further examined in longitudinal studies.

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Keyword: *CyTOF, Cytokines, Radiologically Isolated Syndrome, Biomarker*

#319 Immunosenescence trajectories in diverse human populations: evaluation of immune age towards personalized therapies

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Introduction:

Immunosenecence (ISC) is the natural process of immune aging involving gradual changes in the composition and function of adaptive and innate immune cells, leading to a weakened immunity. Inflammaging is a low-grade inflammation that increases with age. In Multiple Sclerosis (MS), ISC processes may take place prematurely which may contribute to disease progression. Cellular and phenotypic changes are well-established especially within the T cell compartment. Yet, there is a crucial need to assess the impact of genetic variation, sexual-dimorphism and viral infections on ISC trajectories in an ethnically diverse large cohort.

Objective: To establish reference trajectories of physiological ISC that would be evaluated in the MS context.

Approach: Here, we characterized phenotypic, transcriptomic and proteomic profiles in a large-scale manner (n-256, age: 25-88 years) using multiparametric flow cytometry, sc-RNA-seq and CITE-seq.

Results: Cytometric data was empirically clustered using PhenoGraph, resulting in 38 PBMC and 43 T-cell discrete clusters. Linear regression model adjusted for sex, genetic ancestry and CMV seropositivity highlighted age-associated clusters. While sc-RNA-seq revealed 31 distinct PBMCs cell-clusters. Using both approaches, we confirmed earlier observations: decreased naïve CD8 and CD4 T-cells ($p=1.05x10^{-26}$, p=0.0001), and increased CD8 TEMRA ($p=5.93x10^{-15}$). Naïve CD4 were enriched in SOX3, known to regulate thymocyte development. Cytometric data revealed a novel $CD56^{+}PD\text{-}L1^{+}GranzymeB^{+}$NK cell-subset increasing in frequency with age specifically within Hispanics (p=0.001); there was also an increase in frequency of activated IgD^{low}CD4 central memory T-cells (p=0.0079) across non-Hispanic whites (NHW), Hispanics and African American (AA). Unbiased transcriptomic clustering highlighted changes in cytotoxic $CD4^{+}$ CTLT-cells (p=0.009), mucosal associated invariant T-cells (MAIT) (p=0.0013), regulatory T-cells (p=0.006) and dg-T-cells ($p=9.51x10^{-5}$). Three subtypes of MAIT cells, decrease with age. Our data indicate reciprocal ISC trajectory shifts within the MAIT and regulatory T-cell compartments, which may create a permissible environment for tumor development. Finally, we estimated an individual-specific "immunologic age" that captures 58.4% of variance in chronological-age and may be associated with certain brain volumetric measures.

Conclusion: Our data uncovered ISC trajectories in diverse populations. A novel effector cytotoxic $CD56^{+}PD\text{-}L1^{+}GranzymeB^{+}$ NK-cell subtype is reported, perhaps contributing to MS disease progression and higher cancer incidence in elderly. Our findings will contribute to an ongoing effort towards personalized immunotherapies.

Keyword: *immunosenescence, Multiple Sclerosis, NK cells, MAIT Cells, Regulatory T cells*

Learning about neuroimmunology through Parkinson's diseases

#344 Brain-to-gut trafficking of alpha-Synuclein by CD11c+ macrophages in Parkinson's Disease

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INTRODUCTION

Although Parkinson's disease (PD) is classically known as a motor disorder, it is riddled with an assortment of non-motor manifestations including constipation, one of the initial symptoms that patients experience. Investigations into the intestines of PD patients, have revealed the presence of alpha-Synuclein (aSyn) accumulations similar to the detrimental aggregates associated with neurodegeneration in patients' brains. Where these aggregates originate and how they propagate throughout the body, however, remains unclear. Recent focus on the immune system has revealed a role for immune cells in the neurodegeneration in PD. As these cells are capable of traveling throughout the body, they may be the key to aSyn propagation and PD development. Uncovering the role of the immune system in aSyn propagation is crucial for the development of interventions aimed at halting PD progression.

OBJECTIVE

To test if immune cells traffic aSyn from the brain to the intestines in a mouse model of PD.

METHODS

We utilized a mouse model of PD, where an Adeno-associated Virus serotype 1/2 (AAV1/2), expressing a human mutated form of aSyn (haSyn), is injected into the SN of WT mice. As a control, an equal amount of empty AAV (EV) was used. At different time points following injection, the brains and intestines were isolated and evaluated by either immunohistochemistry, flow cytometry, or single cell sequencing.

RESULTS

Localized expression of haSyn in the brain, resulted in increased accumulation of haSyn in the intestines of mice. Interestingly these accumulations were not present in the intestinal ganglia but rather in the CD11c+ macrophages. CD11c+aSyn+ cells could also be found in the brains of both PD patients and animals, suggesting that these cells originated there. Single cell sequencing revealed that the intestines and the brain share a unique population of CD11c+ cells enriched for both migration markers and the aSyn-receptor, LRP1. By developing a novel method to tag the immune cells in the brain, we were able to track the migration of these cells from the brain to the gut of control and PD animals.

CONCLUSIONS

CD11c+ macrophages can take up aSyn accumulations in the brain and traffic them down to the intestines in PD, where they can likewise accumulate and cause disease. Identification of these cells provide a novel target for therapeutic interventions aimed at PD progression prevention.

Keyword: *Neurodegeneration, Parkinson's Disease, Innate Immune system, Gut-Brain Axis*

August 23

CNS barriers

#127 CRTAM-Necl2 interaction at the glia limitans triggers astrogliosis and protection against EAE

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**Equal contribution*

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) where myelin-specific T cells migrate across the blood-brain barrier (BBB) and cause demyelination. Recently, CRTAM was identified as a key molecule in the formation of murine cytotoxic $CD4^+$ T cells. CRTAM interacts with Necl-2, promoting cytotoxic function and vessel-adhesive properties. However, the involvement of $CD4^+CRTAM^+$ T cells and the CRTAM-Necl-2 interaction in MS disease pathology was unknown. Here, we show that $CD4^+CRTAM^+$ T cells are present in MS brain lesions, together with high expression of Necl-2 on astrocyte endfeet. Next, we induced experimental autoimmune encephalomyelitis (EAE) in wild-type and CRTAM-transgenic mice (CRTAM-Tg/Lck), which display T cell-specific CRTAM expression after activation. Surprisingly, only 24% of CRTAM-Tg mice developed EAE symptoms, showing grey matter lesions in the spinal cord, while symptom-free mice acquired anxiety-like behaviour, identified using the open field test. This was associated with increased astrocyte activation in the brain cortex of all CRTAM-Tg/Lck mice. At the time of disease onset in WT mice, CRTAM-Tg/Lck $CD4^+$ T cells exhibited a MOG-reactive, inflammatory phenotype and increased cytotoxic properties. On the other hand, flow cytometric analysis revealed that $CD4^+$ effector T cells were activated in the draining lymph nodes, but did not accumulate in the CNS. This was accompanied by reduced BBB leakage in the spinal cord parenchyma, although IgG and cell infiltrates seemed to accumulate in perivascular spaces, restricted by the glia limitans. In vitro, $CD4^+CRTAM^+$ T cells display an altered crawling phenotype, potentially favouring transmigration. Together, our data suggest that autoreactive CRTAM-Tg/Lck $CD4^+$ T cells are capable of crossing the endothelial cell barrier, but are unable to breach the Necl-2^+ glia limitans and cause CNS damage and full-blown disease after EAE induction. Therefore, we postulate that CRTAM-Necl2 triggering at the glial cell barrier identifies a new mechanism of cell communication, inducing an astrocytic response that restricts immune cell infiltration and propagates astrogliosis in this Tg mouse model.

Keyword: *CD4 T cells, blood brain barrier, multiple sclerosis, EAE*

#243 Cerebral microangiopathy is a key mediator of interferon-alpha neurotoxicity

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Although interferon-alpha is critical for antiviral and immunoregulatory roles, when it is chronically elevated, it causes diseases collectively called interferonopathies. These interferonopathies include genetic disorders like Aicardi-Goutières syndrome (AGS), autoimmune diseases such as CNS-lupus and chronic and congenital viral infections. The neurotoxic effects of interferon-alpha in the brain are poorly understood. This is exemplified by AGS where there is no cure and treatments currently manage symptoms. Although, interferon-alpha affects all cells in the brain, we hypothesised that the vasculature may be a key mediator of disease in AGS.

We investigated this hypothesis using a mouse model that chronically overproduces interferon-

alpha in the brain, termed GIFN mice. Single cell RNA sequencing was used to identify the cell type in the brain most sensitive to interferon-alpha signalling. We validated the role of this cell type by conditionally knocking out the interferon-alpha receptor on this cell type in GIFN mice. Additionally, we verified our findings in brain tissue from patients with AGS.

Single cell RNA sequencing revealed endothelial cells to be the most responsive cell to interferon signalling in the brains of GIFN mice. GIFN endothelial cell transcriptomes suggested presence of mitochondrial dysfunction, inflammasome activation and cell death, immune cell recruitment, and altered angiogenesis. Importantly, absence of the interferon-alpha receptor in endothelial cells of GIFN mice resulted in a significant improvement of many disease features. This included reduced tissue pathology, clinical disease and mortality. Moreover, comparing brain pathology of patients with AGS and GIFN mice, we found close similarities in altered vascular features and phenotype between the mouse model and humans.

These results demonstrates that chronic cerebral interferon-alpha signalling causes vasculopathy in the brain, which drives tissue damage and disease in mice and likely patients. Our findings indicate that targeting the blood vessels offer a promising approach to treating patients with AGS as it is more accessible to therapeutics than the brain parenchyma.

Keyword: *Interferon-alpha, Neurotoxicity, Neuroinflammation, Cerebral angiopathy, Aicardi-Goutières syndrome*

Infection and neuroinflammation

#64 Trichinella spiralis promotes neuroimmune remodeling that ameliorates disease in a mouse model of multiple sclerosis

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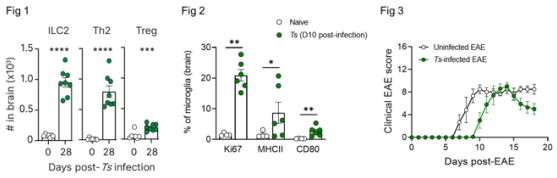
The immune response to helminths is diametrically opposed to the response typically generated during inflammatory autoimmune diseases such as multiple sclerosis (MS). This has provoked investigation into harnessing helminths as immunotherapy for inflammatory diseases. Trichinella spiralis (Ts) is a nematode that can induce neurological symptoms in a subset of severely infected individuals; however, the mechanism by which symptoms are induced remains unclear. Even in mice, little is known about how Ts affects the central nervous system (CNS) following infection, and even less is known about how Ts alters neuroinflammation during MS-like disease. We hypothesized that chronic Ts infection would ameliorate experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, by inducing a type 2 immune response in the CNS to counter the neuroinflammation induced during EAE.

First, we investigated remodeling of the neuroimmune landscape following Ts infection. Time-course infections revealed rapid infiltration of a variety of leukocytes into the brain, including T cells, dendritic cells, and macrophages. Increased cellularity in the brain was long-lasting, with robust populations of T helper 2 (Th2), regulatory T cells (Treg), and type 2 innate lymphoid cells (ILC2) in the brain at 4 weeks post-infection (Fig 1). T cells in the mesenteric lymph node (mLN) over-express integrins necessary to cross the blood-brain barrier (CD49d), suggesting leukocytes may be trafficking from the gut to the

CNS. We postulated that the rapid influx of immune cells would activate CNS-resident cells, especially microglia. Indeed, microglia ($CD45^{lo}CD11b^{+}P2RY12^{+}$) proliferate and up-regulate antigen presentation machinery (MHCII, CD80) shortly after the peak of Ts-induced T cell infiltration in the brain (Fig 2).

To investigate whether these immune alterations protect against EAE, mice were chronically infected with Ts prior to EAE induction. Ts infection delayed disease onset and induced a striking remission of symptoms that is not observed in uninfected controls (Fig 3). During the priming phase of EAE, Ts-infected mice had increased populations of Th2-skewed ($IL\text{-}13^{+}$, $GATA3^{+}$) myelin-reactive $CD4^{+}$ T cells in the periphery. T cells primed in a Ts-infected host were impaired in their ability to transfer disease to uninfected recipients in an adoptive transfer model of EAE.

These data highlight a previously unappreciated role of Ts in modulating the neuroimmune environment. Ts induces chronic changes to the CNS immune landscape that protects from downstream neuroinflammatory insult and promotes remission potentially via microglia-Th2 interactions. These findings may provide insight into immune pathways that can be harnessed for therapeutics in neuroinflammatory autoimmune disease.



Keyword: *multiple sclerosis, helminth infection, T cells, microglia, experimental autoimmune encephalomyelitis*

#330 Sepsis, complement, and microglial synaptic pruning: findings in the mouse and human brain

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Sepsis is associated with brain dysfunction, including cognitive impairment and dementia, especially in the elderly. However, the mechanisms driving this brain dysfunction are unclear. We conducted transcriptomic analyses in both human and mouse brain tissue after sepsis to explore potential mechanisms. Bulk RNA-seq was completed on sex-matched postmortem parietal cortex samples from 12 patients who died of sepsis or nonsepsis causes from the Adult Changes in Thought (ACT) brain bank (Bustamante et al. 2020, DOI:10.1164/rccm.201909-1713LE). We also conducted bulk RNA-seq on whole mouse brains 5-and 25- days following cecal ligation and puncture (CLP), a model of abdominal sepsis. In all datasets, we found that complement system genes, among other neuroimmune pathways, were differentially expressed in the sepsis groups. Furthermore, weighted gene coexpression network analysis (WGCNA) of the human dataset revealed a correlation ($\rho = 0.41$, $P = 0.05$) between sepsis and a module related to synaptic function.

To investigate the cellular origins of broad transcriptomic changes, we did single nucleus RNA-seq on a subset of 8 sex-matched brain samples used in the original bulk RNA-seq analysis. Cytokine signaling (e.g., TNFRSF1A,

TLR4) and complement system (e.g., C1QB, C1QC, C5AR1) genes were differentially expressed in microglia and astrocytes in the sepsis group. Additionally, gene ontology analysis using ShinyGO of microglial differentially expressed genes revealed synapse disassembly (GO:0098883) to be a cellular process altered in microglia.

The complement system regulates both neuroinflammation and synapses in the brain. Microglia prune synapses tagged with complement proteins. Thus, we investigated complement-driven, microglial-mediated synaptic pruning following sepsis in our mouse models. We measured microglia synaptic phagocytosis by flow cytometry as proportion of microglia ($CD11b^{+}/CD45^{mid}$) positive for synaptic protein (SNAP25). Additionally, given that amyloid pathology is present in many cognitively normal aged individuals, we investigated whether amyloid pathology accelerates sepsis-induced brain dysfunction. We modeled asymptomatic amyloid pathology with 4–5-month-old C57BL/6 5xFAD mice. Wild-type mice had no changes to microglial synaptic phagocytosis following sepsis. However, 5xFAD sepsis survivors had increased levels of microglial synaptic phagocytosis relative to 5xFAD controls acutely and long term (5-days and 6-weeks post CLP). We used both males and females but are currently underpowered to assess sex differences.

Sepsis was associated with an increase in brain expression of complement genes in both humans and mice. In patients with vulnerable brains, pruning of synapses by microglia may be a mechanism of sepsis-induced brain dysfunction due to increased levels of complement signaling.

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Keyword: *Sepsis, Microglia, Synaptic pruning, Transcriptomics, Alzheimer's disease*

Peripheral Neuroimmunology

#13 Multimodal control of dendritic cell functions by nociceptors

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Interactions between nociceptors and dendritic cells (DCs) can modulate immune responses in barrier tissues, however, our understanding of the underlying communication frameworks remains rudimentary. Here, using a combination of in vitro and in vivo approaches, we show that nociceptors, in a context-dependent manner, control DC functions in three molecularly distinct ways: First, upon exposure to noxious stimuli, nociceptors release the calcitonin gene–related peptide that imparts a distinct transcriptional profile on steady-state DCs characterized by expression of pro–IL-1b and other genes implicated in DC sentinel functions, but no overt DC activation or proinflammatory cytokine secretion. Second, upon concomitant exposure to noxious and immune stimuli, nociceptor activation induces contact-dependent calcium

fluxes and membrane depolarization in DCs and enhances their production of proinflammatory cytokines. Finally, nociceptor-derived chemokine CCL2 serves as a DC retention signal and contributes to the orchestration of DC-dependent local inflammation and the induction of adaptive responses against skin-acquired antigens.

Thus, the combined actions of nociceptor-derived chemokines, neuropeptides, and electrical activity fine-tune DC responses in barrier tissues.

Keyword: *Dendritic cells, Nociceptors, Peripheral neuroimmunology*

B cells in Neuroinflammation

#345 T:B cell communication in ectopic lymphoid follicles in CNS autoimmunity

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Meningeal ectopic lymphoid follicle-like structures (eLFs) have been described in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), but the cellular processes within and their role in CNS autoimmunity is not clearly understood. Here, we employed the Th17 adoptive transfer EAE model, which features formation of large, numerous eLFs, to analyze the cellular processes within these structures. We show that clusters of activated B cells and B1/Marginal Zone-like B cells are overrepresented in the CNS and identified B cells poised for undergoing antigen-driven germinal center (GC) reactions and clonal expansion in the CNS. Furthermore, we found evidence for enhanced capacity for antigen presentation and immunological synapse formation in CNS B cells. To visualize Th17:B cell communication in eLFs, we labeled Th17 cells with a ratiometric calcium sensor, allowing us to study their interactions with tdTomato-labeled B cells in real-time using intravital microscopy of the CNS. Thus, we demonstrate for the first time that there is extensive communication and long-lasting contacts between T and B cells in meningeal eLFs, and that B cells are able to reactivate T cells. Consistent with these findings, we show that CNS T cells depend on CNS B cells to maintain a highly pro-inflammatory cytokine profile. Our data suggest that interaction of T and B cells in meningeal eLFs in our model not only promotes differentiation and clonal expansion of B cells, but also leads to reactivation of CNS T cells and thereby supports smoldering inflammatory processes within the CNS. Thus, our results may provide a direction for future research into the function of eLFs in MS.

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Keyword: *ectopic lymphoid follicles, tertiary lymphoid structures, meninges, Th17 cells, B cells*

#44 T-bet+ Memory B Cells Induce Disease Relapses in Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is a neurodegenerative disorder driven in part by immune cell mediated damage to oligodendrocytes and neurons. The efficacy of anti-CD20 monoclonal antibodies in treating MS has demonstrated the importance of B cells in driving disease pathology. B cell subsets exhibit tremendous diversity; however, no subset has yet been identified as pathogenic in MS, though evidence suggests that a memory B cell subset is important. One memory B cell subset, which expresses the T-bet transcription factor, has been found to be increased in the blood and central nervous system (CNS) of MS patients, suggestive of its pathogenicity. We developed an in vitro polarization protocol to differentiate naïve B cells extracted from mice into memory B cells that do or do not express T-bet. To test their pathogenicity, we transferred either naïve B cells, T-bet$^-$ memory B cells, or T-bet$^+$ memory B cells into mice with ongoing experimental autoimmune encephalomyelitis, a model of CNS autoimmunity, and then evaluated CNS pathology using flow cytometry and histology. Transfer of T-bet$^+$ memory B cells, but not other B cell subsets, was associated with increased disease severity during a relapse after the initial peak of disease in recipients, as well as with increased numbers of B cells, CD4$^+$ T cells, and peripheral macrophages entering the CNS. By transferring B cells from red fluorescent protein positive animals, we determined that T-bet$^+$ memory B cells preferentially accumulate in the CNS relative to naive B cells and their accumulation is correlated with MHC2 and CD40 expression on macrophages, implying macrophage activation. In vitro testing was used to determine if T-bet$^+$ memory B cells are causally responsible for macrophage recruitment into and activation within the CNS. Bone marrow derived macrophages (BMDMs) were placed on top of transwell filters and supernatants from naïve, T-bet$^-$ memory, or T-bet$^+$ memory B cell cultures were placed underneath and the number of BMDMs migrating to the underside of the filters was quantified. Culture supernatants from T-bet$^+$ memory B cells, but not other subsets, induced BMDM migration. Similarly, incubation of BMDMs with supernatants from T-bet$^+$ memory B cells, but not other subsets, could induce CD40 expression as determined by flow cytometry. These results suggest that T-bet$^+$ memory B cells are a pathogenic subset and can accumulate in the CNS during EAE, where they recruit inflammatory macrophages into the CNS.

Keyword: *B cells, T-bet, Macrophage, EAE, Multiple Sclerosis*

#55 B cell depletion with anti-CD20 promotes neuroprotection in a BAFF-dependent manner in mice

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Anti-CD20 therapy is highly efficacious in preventing new white matter lesions in relapsing-remitting MS patients, however its capacity to protect against grey matter injury and axonal damage is unclear. Anti-CD20 treatment in humans is associated with an increase in the B cell activating factor (BAFF), presumably through the removal of B cells – the main consumers of BAFF. Anti-CD20 treatment spares most terminally-differentiated antibody-secreting plasma cells, including immunoglobulin A (IgA)-secreting plasma cells which our lab has shown to have immunoregulatory functions. In addition, BAFF signaling through the BAFF-receptor also

promotes neuronal survival in other neurodegenerative contexts.

Using a passive model of experimental autoimmune encephalomyelitis (EAE) whereby adoptively transferred $PLP_{139-151}$-primed Th17 cells induce brain leptomeningeal fibroblast remodeling and tertiary lymphoid tissue formation, we found that prophylactic monoclonal anti-CD20 (18B12) treatment is highly efficacious in sparing myelin content, reducing myeloid cell activation and mitochondrial stress, and preventing axonal damage in the brain subpial grey matter. Anti-CD20 treatment led to an increase in the B cell survival factor (BAFF) in the serum, cerebrospinal fluid (CSF), and leptomeninges, and serum BAFF levels positively correlated with subpial grey matter myelin content in the brain. Co-treatment of anti-CD20 with a BAFF-neutralizing monoclonal antibody (Sandy-2) abrogated anti-CD20 efficacy in preventing cortical pathology. While anti-CD20 treatment reduced B cell numbers, we observed an increase in serum immunoglobulin A (IgA) and splenic IgA^+ plasma cells.

Taken together, we postulate that the mode of action of anti-CD20 therapy in MS patients may be multifaceted, including a neuroprotective effect of BAFF on grey matter in collaboration with promoting immunoregulatory plasma cells.

Keyword: *anti-CD20, multiple sclerosis, experimental autoimmune encephalomyelitis, autoimmune neuroinflammation, B cell depletion*

August 24

Emerging therapies for neuroinflammatory conditions

#340 Peptide-coupled RBCs as treatment for autoimmune diseases – dissecting the mechanisms of immune tolerance induction

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Induction of antigen-specific immune tolerance is one of the most specific ways of reverting the abnormal autoimmune reactions. Our group has developed an antigen-specific therapy for the treatment of Multiple Sclerosis (MS), which involves the coupling of autologous red blood cells with a cocktail of seven MS immunodominant peptides. A recent phase Ib clinical trial in MS patients demonstrated its safety and tolerability and provided evidence for immune tolerance induction. Prior data suggest the presence of an indirect mechanism where peptide-coupled red blood cells (pcRBC) are phagocytosed and processed by liver and spleen macrophages, which present the antigens in a tolerogenic way. We have established a reliable protocol for the in-situ digestion of mouse liver and the subsequent isolation and characterization of liver non-parenchymal cells with flow cytometry. We have identified a CD11b+F4/80+ cell population, other than the CD11b+F4/80hi tissue-resident macrophages (Kupffer cells, KC), which appears in mouse liver after intravenous injection of pcRBC and coincides with an increase in monocyte and a decrease in KC numbers. While all three populations are able to uptake pcRBC, KC are the predominant phagocytosing population and seem to acquire a tolerogenic phenotype. Aiming to address the mechanisms involved in pcRBC uptake, we excluded the involvement of the

complement system, as C3 knockout animals showed comparable levels of phagocytosed pcRBC to their wildtype littermates. Lastly, we explored the tolerization potential of KC-depleted animals in Experimental Autoimmune Encephalomyelitis through pcRBC and demonstrated that KC are indispensable for tolerance induction. Collectively, we have successfully established a model system for the study of pcRBC-mediated immune tolerance and have identified the involvement of KC in pcRBC phagocytosis and tolerance induction. Moving forward, our focus will lie on the in-depth phenotypical and transcriptional characterization of the phagocytosing populations, ultimately aiming to elucidate the establishment of pcRBC-induced immune tolerance.

Keyword: *Multiple Sclerosis, therapeutics, immune tolerance, peptide-coupled cells, EAE*

#156 Peptide-coupled RBCs as treatment for autoimmune diseases – dissecting the mechanisms of immune tolerance induction

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Neurodegenerative diseases are among the top causes of death with no known cures. Neurodegenerative diseases continue to increase in the expanding elderly population, indicating a critical need for the development of treatments and preventive strategies. Inflammation has been identified as a precursor of neurodegenerative disease and has been shown to increase with age, a process known as inflammaging. Activation of the inflammasome has been shown to contribute to inflammaging. The inflammasome is a multiprotein complex of the innate immune response that processes the cytokines pro-interleukin (IL)-1beta and pro-IL-18 into their active forms. The inflammasome consists of a sensor protein such as NOD-like receptor protein 1 (NLRP1), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1. Here, we examine the levels of inflammasome activation in aged mice with treatment of a monoclonal antibody against ASC (anti-ASC). We also examine the effects of extracellular ASC specks on microglia. We analyzed cortical lysates from young (3 month), aged saline-treated (18 month), and aged anti-ASC-treated (18 month) mice for the expression of inflammasome proteins. Our data indicate that protein levels of NLRP1, ASC, caspase-1, caspase-8, and IL-1beta were elevated in the cortex of aged mice, and that anti-ASC decreased the expression of these proteins. Additionally, these proteins form a novel NLRP1-caspase-8 non-canonical inflammasome comprised of NLRP1, caspase-8, and ASC. Moreover, we administered ASC specks to microglial cells in vitro. We show microglia can uptake extracellular ASC specks. Furthermore, we measured caspase-1 activity and lactate dehydrogenase (LDH) release in the cell media to determine inflammasome activity and cell death, respectively. We found that at high concentrations, ASC specks significantly activate the inflammasome and lead to cell death. Together, these data show that the inflammasome, specifically ASC specks, contribute to brain inflammaging. Thus, anti-ASC may be a potential therapeutic for the amelioration of inflammasome-mediated inflammaging in the central nervous system, which could potentially be used to prevent neurodegenerative diseases.

Keyword: *Inflammasome, Inflammaging*

#299 Nasal anti-CD3 mAb induces Tregs that dampen microglial activation and treat neuroinflammatory diseases including MS, AD and ALS.

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In an animal model of progressive MS, we found that nasal anti-CD3 mAb induced IL-10 dependent Tregs that dampened microglial and astrocyte inflammation, reduced myelin and axonal damage and ameliorated clinical disease (Mayo, Brain 2016). We have translated this to humans and in a dose-ranging study found that nasal administration of a fully human mAb (Foralumab) given for 5-10 days modulated immune function in healthy volunteers (Chitnis, Front Immunol, 2022) and treated active COVID by reducing lung inflammation and serum markers of inflammation (Moreira, Front Immunol, 2021). Moreover, nasal Foralumab downregulated NKG7 and increased TGFB1 and GIMAP7 expression in T cells in healthy subjects and subjects with MS and COVID-19 (Moreira PNAS 2023). We have now treated 6 subjects with non-active progressive MS with nasal Foralumab given for up to a year in 3 week cycles (2 weeks Rx/ one week holiday) and observed decreased microglial activation as measured by [F-18] PBRO6 microglial PET imaging, clinical stabilization of disease and immune modulation. No toxicities were observed. A double-blind placebo-controlled trial is planned. We have also found decreased microglial activation and amelioration of disease in animal models of Alzheimer's disease, ALS and traumatic brain injury treated with nasal anti-CD3. Human trials in these diseases are being planned. Nasal administration of anti-CD3 mAb represents a novel approach to treat neuroinflammatory and neurodegenerative diseases by inducing Tregs and modulating the immune system to dampen microglial activation.

Keyword: *Immunomodulation, Microglia, Tregs, progressive MS, Alzheimer's disease*

Influence of diet and microbiota on neuroinflammation

#121 Ketogenic diet promotes social stress resistance and modifies microglial morphology and ultrastructure in male mice

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Ketogenic diet (KD) is high-fat, low-carbohydrate diet which may promote stress resilience. Microglia are the resident immune cells of the brain; they coordinate brain immune responses and can react to psychological stress and mediate stress-related brain changes. This study focused on the effect of KD, its relationship to stress resilience and its effect on microglia. Two-month-old adult male C57BL/6 mice received KD versus control diet (CD) starting 4 weeks prior to the

experiment. The dietary effects on the response to chronic stress were investigated by comparing non-stressed controls with animals undergoing 10 days of repeated social defeat (RSD). After RSD, mice were classified as resilient or susceptible to stress based on a social interaction test. KD increased the proportion of resilient mice compared to CD. We studied the underlying mechanisms by focusing on microglia in the ventral hippocampus, a region affected by chronic stress. Using TMEM119/IBA1 double staining, we found that KD does not affect microglial number or distribution. However, changes in their soma and arborization area were linked to the effect of diet and stress. Ultrastructural analysis of microglia by electron microscopy showed that KD reduced the number of markers of cellular stress. Microglia also displayed general effects of stress in the number of contacts with synaptic elements. Lipidomic analysis of hippocampus showed distinct lipidomic signatures. This study provides valuable results regarding the dynamic relationship of diet-induced energetic shifts, psychological stress, and microglia.

Keyword: *microglia, ketogenic diet, stress, electron microscopy, stress resilience*

#284 Identification of commensal gut microbiota signatures as predictors of clinical severity and disease progression in multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system and a leading cause of neurological disability in young adults. Clinical presentation and disease course are highly heterogeneous. Typically, disease progression occurs over time and is characterized by the gradual accumulation of disability. The risk of developing MS is driven by complex interactions between genetic and environmental factors, including the gut microbiome. How the commensal gut microbiota impacts disease severity and progression over time remains unknown.

Methods: In a longitudinal study, disability status and associated clinical features in 60 MS patients were tracked over 4.2 ± 0.97 years, and baseline fecal gut microbiome was characterized via 16S amplicon sequencing. Progressor status, defined as patients with an increase in Expanded Disability Status Scale (EDSS), were correlated with features of the gut microbiome to determine candidate microbiota associated with risk of MS disease progression.

Results: We found no overt differences in microbial community diversity and overall structure between MS patients exhibiting disease progression and non-progressors. However, a total of 45 bacterial species were associated with worsening disease, including a marked depletion in Akkermansia, Lachnospiraceae, and Oscillospiraceae, with an expansion of Alloprevotella, Prevotella-9, and Rhodospirillales. Analysis of the metabolic potential of the inferred metagenome from taxa associated with progression revealed a significant enrichment in oxidative stress-inducing aerobic respiration at the expense of microbial vitamin K_2 production (linked to Akkermansia), and a depletion in SCFA metabolism (linked to Lachnospiraceae and Oscillospiraceae). Further, statistical modeling demonstrated that microbiota composition and clinical features were sufficient to robustly

predict disease progression. Additionally, we found that constipation, a frequent gastrointestinal comorbidity among MS patients, exhibited a divergent microbial signature compared with progressor status.

Conclusions: These results demonstrate the utility of longitudinally assessing disease severity in highly heterogenous MS cohorts to identify unique baseline gut microbial signatures predictive of disease progression.

#119 Regulation of the antiviral immune response by the sensory nervous system

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Host protection depends on its ability to resist infection by microorganisms. The skin is one of the first lines of defense against pathogens because it contains many immune cells involved in both innate and adaptive immune responses. These cells act as sentinels that are activated when an infection occurs and trigger an inflammatory response by recruiting more effector cells. Pain is one of the main signs of inflammation. Indeed, the skin is densely innervated by nociceptive sensory neurons that can be activated by a wide variety of inflammatory mediators and pathogens, resulting in a painful sensation. For many years, host protection against infection appeared to depend almost exclusively on the immune response, but recent studies have shown that the sensory nervous system plays a critical role in infectious disease. When activated, nociceptive sensory neurons release mediators locally in the skin that can modulate the immune cell response. This neuroimmune regulation may have proinflammatory or anti-inflammatory properties, suggesting that its regulatory role depends on the pathological context under investigation. Nociception has long been known to be modulated in bacterial and fungal infections, but nothing has been known about viral infections of the skin. We have previously shown that $Nav_{1.8}^{+}$ sensory neurons are required for the down-regulation of neutrophil infiltration in skin to limit the severity of tissue damage, as well as for eliciting a robust antiviral response by cytotoxic $CD8^{+}$ T cells in a model of cutaneous herpesvirus simplex-1 (HSV-1) infection. These first findings paved the way for more detailed studies of the molecular mechanisms involved. Our recent studies have revealed tissue- and neuropeptide-specific regulation of HSV-1 infection by sensory neurons in both skin and dorsal root ganglia (DRG) : two sites of HSV-1 replication. This allowed us to describe a novel neuroimmune signaling pathway at the cellular and molecular levels that occurs after cutaneous HSV-1 infection. Overall, our data demonstrate that the sensory nervous system plays an important role in regulating both innate and adaptive immune responses to viral infection, opening up possibilities for novel therapeutic strategies.

Keyword: *Sensory neurons, HSV-1, Inflammation, Skin, Dorsal root ganglia (DRG)*

CNS-infiltrating innate immune cells

#124 Essential cytokines driving macrophage effector phenotypes in neuroinflammation

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Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS), where tissue damage is caused by infiltrating peripheral immune cells. Mononuclear phagocytes are the most abundant cell type in MS lesions, where they can play crucial roles both for tissue damage and repair through a spectrum of effector phenotypes. However, we lack a systematic understanding of how different phagocyte effector phenotypes are specified in the context of neuroinflammatory lesions. In this work, we first established a new method to conduct in vivo CRISPR KO screens in macrophages. We then conducted systematic CRISPR KO screens targeting cytokine receptors and downstream signaling genes in different Experimental Autoimmune Encephalomyelitis (EAE) models. We aimed to detect essential cytokines driving the specification of destructive (M-iNOS, defined by inducible Nitric Oxide Synthase (iNOS) expression) and reparative (M-Arg1, defined by Arginase 1 expression) phenotypes. We were able to identify and further validate IFNγ and TNFα as the main cytokines essential for M-iNOS specification, and TGFβ and GM-CSF as the main cytokines essential for M-Arg1 specification. Finally, through single cell sequencing of wild-type and IFNγ, TNFα, TGFβ and GM-CSF receptor KO macrophage populations from EAE lesions, we characterized in depth the contribution of each cytokine to the molecular phenotype of macrophages subpopulations. Our work thus untangles the cytokine signaling network regulating macrophage phenotypes in neuroinflammatory lesions, and provides an important methodological advance for the further study of macrophage functions in the context of neuroinflammatory conditions like MS.

Keyword: *Innate immunity, Neuroinflammation, CRISPR screen, scRNAseq*

#140 Lipid metabolism directs the phenotype of foamy phagocytes in multiple sclerosis lesions

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Infiltrated macrophages and resident microglia play a central role in the pathology of demyelinating diseases such as multiple sclerosis (MS). They promote lesion development by stimulating neuroinflammation, demyelination, and neurodegeneration by releasing inflammatory and toxic mediators. However, phagocytes can also adopt a lesion-resolving phenotype that facilitates remyelination through the secretion of anti-inflammatory and neurotrophic factors, and by the clearance of myelin debris. Our findings show that the lipid load of macrophages and microglia is a major determinant of their reparative potential. Phagocytosis of myelin initially reduces the

inflammatory activity of phagocytes. However, continuous accumulation of myelin-derived lipids eventually blunts their protective features, and induces an inflammatory transcriptional profile. Myelin loaded phagocytes show alterations in lipophagy, lipolysis and fatty acid metabolism that promote foam cell formation. Enhancing lipid efflux from foamy phagocytes resolves inflammation and stimulates remyelination. Hence, targeting lipid metabolism in macrophages and microglia provides a promising strategy to induce repair in the central nervous system.

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Keyword: *phagocytes, remyelination, lipid metabolism*

#56 Depletion of leptomeningeal neutrophils ameliorates age-dependent grey matter demyelination

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Background: People living with multiple sclerosis (MS) experience episodic central nervous system (CNS) white matter lesions instigated by autoreactive T cells. With age, MS patients show evidence of grey matter demyelination and experience devastating non-remitting symptomology. What drives progression is unclear and has been hampered by the lack of suitable animal models. We have recently published that passive experimental autoimmune encephalomyelitis (EAE) induced by an adoptive transfer (A/T) of young Th17 cells induces a non-remitting clinical phenotype that is associated with persistent leptomeningeal inflammation and cortical pathology in old, but not young SJL/J recipient mice[1]. While the quantity and quality of T cells did not differ in the brains or leptomeninges of old vs young EAE mice, an increase in leptomeningeal neutrophils was observed in old mice. Using single-cell RNA sequencing, we have also observed the neutrophil cluster from old EAE mice show upregulation of genes associated with inflammation and antigen presentation. To corroborate this in humans, neutrophils were also found in the leptomeninges of a subset of progressive MS patient brains that showed evidence of leptomeningeal inflammation concomitant with subpial cortical demyelination. Recent unpublished work continuing this line of inquiry has shown that neutrophils from old EAE mice express higher levels of MHCII and

costimulatory molecules CD80 and CD86 by flow cytometry, indicating a potential for supporting T cell proliferation. Moreover, systemic treatment with a neutrophil depleting antibody leads to lower clinical scores only in old A/T EAE mice along with a reduction in pathology in post-mortem brain tissue.

Methods: Young (6-8 weeks) and old (10-12 months) SJL/J mice were given 10 million PLP-primed Th17 cells i.p. to induce EAE and followed for 11 or 25 days post-induction. Mice were treated prophylactically or therapeutically with a 100µg of neutrophil-depleting anti-Gr1 antibody (RB8-8C5) or isotype control for 7-8 consecutive days to assess impact on clinical manifestation. Brains were collected after sacrifice and embedded in paraffin for histology readouts.

Results: Anti-Gr1 treatment is specific to $Ly6G^+$ neutrophils in the periphery and in the leptomeninges, as $Ly6G^-Ly6C^+$ monocytes remain unaffected after treatment. Neutrophil depletion is effective for up to eight days post-treatment and loses efficacy afterward as mice develop resistance. Old mice treated with anti-Gr1 exhibit lower clinical scores while depletion is effective, and post-mortem analysis of brain cortical regions reveals smaller leptomeningeal immune cell aggregation along with ameliorated grey matter demyelination and microglia activation.

Conclusion: Neutrophils may play an important role in mediating persistent leptomeningeal inflammation and associated CNS pathologies. Deciphering the underlying mechanisms of cortical damage associated with neutrophil infiltration could lead to discovery of novel disease-modifying therapies for progressive MS patients.

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Keyword: *neutrophil, inflammation, leptomeninges, cortex, neuropathology*

Immuno-neuro-oncology

#285 Antiviral T cells populate glioblastoma and originate from pre-existing brain resident memory T cells

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Memory T cells specific for common viral infections populate human brain tumors and are often present in high frequencies. Interestingly, these cells express surface markers, CD69 and CD103, indicative of tissue residency. In healthy and malignant peripheral tissues, resident memory T cells (T_{RM}) can provide long-term protection against reinfections or cancers, however their role in the context of the brain and brain tumors is not fully understood. Glioblastoma (GBM) is an aggressive, invariably fatal brain tumor and immunotherapies which have been revolutionary in the treatment of other cancers have failed to impact patient survival. A better understanding of the unique brain tumor immune environment is needed. Moreover, surveillance of tumors by bystander (non-tumor specific) memory T cells is understudied despite their potential to contribute to anti-tumor immunity. Indeed, the source of these virus-specific memory T cells (vsT_M) is unknown; they may originate from circulating memory T cells (T_{CircM}) that infiltrate

the tumor, and/or pre-existing T_{RM} that were in the brain prior to tumor formation. Here, we leverage a mouse model of an acute viral infection to study the migration dynamics and origin of antiviral memory T cells in GBM by establishing mouse models with distinct memory T cell compartments. In mice lacking T_{RM}, we found that a small population of T_{CircM} can infiltrate tumors and surprisingly upregulate CD69, but not CD103. In addition to being a marker of residency, CD69 can also be upregulated upon T cell activation directly through antigen binding or indirectly by inflammatory cytokines. As viral antigen is absent in the tumor, ongoing work will assess the resident nature of this population and the mechanism of CD69 upregulation. To assess the contribution of pre-existing T_{RM} to vsT_M in GBM, we established a mouse model lacking T_{CircM}. We found that pre-existing T_{RM} can contribute to tumor vsT_M without help from circulating cells. These cells express both CD69 and CD103, suggesting that pre-existing T_{RM} are the main source of tumor vsT_M in mice. This has immediate implications for our understanding of the T cell repertoire in healthy human brain and we are working to understand the relationship between virus-specific T cells in human brain tumors and peri-adjacent brain. Our findings will broaden our understanding of GBM and brain immunosurveillance, and provide valuable insights for the development of novel T cell-based immunotherapies.

#180 Analysis of factors that limit immunogenicity of intracranial melanomas

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The presence of CD8 T cells (TIL) in cutaneous melanoma correlates with longer survival. However, intracranial (IC) melanomas are poorly infiltrated, resulting in worse patient prognosis. To understand the basis for this, we analyzed murine B16 melanomas implanted subcutaneously (SC) or IC. IC tumors had fewer TIL than SC tumors and were larger. However, adoptively transferred exogenously activated effector CD8 T cells were present in similar numbers in both SC and IC tumors. This suggests that limited immune cell presence in IC tumors is not due to a particular vascular phenotype, but instead, IC tumors do not elicit a robust immune response.

We next used zsGreen$^+$ tumor cells to evaluate antigen (Ag) acquisition, activation/maturation, and movement to tumor draining lymph nodes (tdLN) by antigen presenting cells (APC). Compared to SC tumors, a smaller fraction of Macrophages and Dendritic Cells (DC) in IC tumors had acquired Ag, and the amount of Ag acquired was lower. They were also less activated/mature. Microglia in IC tumors also acquired tumor Ag, but this did not diminish either Ag acquisition or maturation/activation of macrophages and DC. A significant fraction of DC in SC and IC tdLN (axillary (aLN) and deep cervical (dcLN), respectively) and meninges were Ag positive (Ag$^+$). We also found that drainage of intra-cisterna magna (i.c.m.) injected beads to dcLN in IC tumor bearing mice was not altered, demonstrating that meningeal lymphatic vessel drainage is not influenced by the tumor presence. This suggests that dcLN and/or meninges are the primary site(s) of tumor Ag drainage and of T cell activation in IC tumor bearing mice. However, the absolute numbers of Ag$^+$ DC in IC tumor draining dcLN and meninges were substantially lower than the number in SC tumor draining aLN, consistent

with the fact that the dcLN is substantially smaller. To date, we have not been able to directly demonstrate the activation of naïve T cells transferred intravenously (IV) or directly i.c.m. in tdLN or meninges in either IC or SC tumor bearing mice. Nonetheless, our data suggests that poor IC melanoma immunogenicity is not due to a vascular phenotype that limits T cell entry but instead is due to (1) a brain microenvironment (not microglia) that limits Macrophages and DC activation/maturation; (2) drainage sites whose small size limits the number of Ag+ DC and of infiltrating T cells available to support an immune response.

Keyword: *APC, Antigen drainage, T cell activation*

Novel approaches for neuroimmunologists

#126 Multidimensional single-cell analysis of meningeal inflammation and cortical microglia in progressive multiple sclerosis

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Meningeal inflammation strongly associates with demyelination and neuronal loss in the underlying cortex of progressive multiple sclerosis (MS) patients, thereby contributing significantly to clinical disability. We and others have shown a crucial role for meningeal inflammation and cortical microglia in driving cortical neurodegeneration. However, which exact meningeal immune cells drive this process is still unknown.

To understand meningeal inflammation-mediated pathology at a single-cell level, we studied cortical microglia and meningeal immune cells in a cohort of post-mortem tissue from MS donors and controls using complementary techniques: single-nucleus RNA sequencing (snRNAseq), multiplex immunofluorescence (mIHC) and imaging mass cytometry (IMC).

With snRNAseq, we identified 10 meningeal immune cell populations, with antibody-secreting B cells (ASC) being the most enriched cell population in the meninges of MS donors, which was completely absent in controls. We also defined 11 subclusters of microglia, 4 of which were enriched in MS cortex samples. In addition, cortical areas in MS donors where microglia were in close contact with neurons and neurodegeneration was limited, contained microglia subclusters enriched for both homeostatic and phagocytic genes. Furthermore, the amount of meningeal ASC correlated negatively with a subcluster of microglia enriched in complement and mitotic genes. More fine-grained analyses of snRNAseq and IMC to study in-depth these immune interactions and how it correlates to pathology are in progress.

With complementary high-resolution -omics techniques, we provide better insight into the inflammatory processes that drive neurodegeneration in progressive MS. Thereby,

we expect to provide new targets for a much-needed therapeutic intervention for progressive MS.

#41 Classifying flow cytometry data using bayesian analysis helps to distinguish ALS patients from healthy controls

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Introduction: Given its wide availability and cost-effectiveness, multidimensional flow cytometry (mFC) became a core method in the field of immunology allowing for the analysis of a broad range of individual cells providing insights into cell subset composition, cellular behavior, and cell-to-cell interactions. Formerly, the analysis of mFC data solely relied on manual gating strategies. With the advent of novel computational approaches, (semi-)automated gating strategies and analysis tools complemented manual approaches.

Methods: Using Bayesian network analysis, we developed a mathematical model for the dependencies of different obtained mFC markers. The algorithm creates a Bayesian network that is HC tree when including raw, ungated mFC data of a randomly selected control cohort (HC). The HC tree is used to classify whether the observed marker distribution (either patients with amyotrophic lateral sclerosis (ALS) or HC patients) is predicted. The relative number of cells where the probability q is equal to zero is calculated reflecting the similarity in the marker distribution between a randomly chosen mFC file (ALS or HC) and the HC tree.

Results: Including mFC data from 68 ALS and 35 HC, the algorithm could correctly identify 64/68 ALS. Tuning of parameters revealed that the combination of 7 markers, 200 bins, and 20 patients achieved the highest AUC on a significance level of $p < 0.0001$. The markers CD4 and CD38 showed the highest zero probability. We successfully validated our approach by including a second, independent ALS and HC cohort (55 ALS and 30 HC). In this case, all ALS were correctly identified and side scatter and CD20 yielded the highest zero probability. Finally, both datasets were analyzed by the commercially available algorithm 'Citrus', which indicated superior ability of the Bayesian network analysis when including raw, ungated mFC data.

Discussion: Bayesian network analysis might present a novel approach for classifying mFC data, which does not rely on reduction techniques, thus, allowing to retain information on the entire dataset. Future studies will have to assess the performance when discriminating clinically relevant differential diagnoses to evaluate the complementary diagnostic benefit of Bayesian network analysis as part of the clinical routine workup.

#274 Development of a Human Microglia Engraftment Model for the Study of Neurodegenerative and Neuroinflammatory Diseases

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Microglia have a critical role in shaping the response to central nervous system (CNS) injury and infection. They can contribute to both protection and pathogenesis in a myriad of neurological disorders, including Alzheimer's Disease (AD), multiple sclerosis, and ischemic stroke. While many studies have identified regulatory factors of microglia in murine models, direct analysis of human microglia is limited to static snapshots of pathogenesis within post-mortem samples, primarily from patients that had clinically advanced disease. There is a critical need for models of human microglia activation that are rigorous and reproducible, yet directly relevant to human disease. Human microglia engraftment of humanized mice will aid in the identification of therapeutic targets and help bridge the gap between bench and bedside. The immunodeficient NSG mouse strain supports human hematopoietic engraftment in the periphery following the transfer of hematopoietic stem cells (HSCs) into sublethally irradiated newborn mice. To support the engraftment of human microglia, we introduced transgenes for either human interleukin 34 (hIL34) under the control of the ENO2 promoter, or the intact locus for human colony stimulating factor 1 (hCSF1) into NSG mice. Following the transfer of CD34+ HSCs, isolated from umbilical cord blood, into NSG-hIL34 or NSG-hCSF1 mice, immunohistochemical and flow cytometric analysis showed efficient and stable human microglia engraftment in the brain cortex, hippocampus, and thalamus. We found no significant difference in peripheral hematopoietic cell populations compared to engrafted NSG controls. These data demonstrate this is a feasible strategy for studying human microglia in vivo. Ongoing studies are focused on characterizing the microglia phenotypes in each transgenic strain and implementing this engraftment strategy in genetic models of neurodegeneration and neuroinflammation. Our model of human microglia engraftment has widespread applications in the study of human neurological disease pathogenesis and the testing of novel therapeutic strategies.

Keyword: *Humanized Mice, Microglia, Hematopoetic stem cells, Central nervous system*

#322 Comparative analysis of methods to reduce activation signature gene expression in peripheral blood mononuclear cells

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Preserving the in vivo transcriptomic profile is essential for accurate cell profiling. Yet, the transcriptomic profiles of cells can rapidly shift once they are removed from in vivo conditions, particularly in the case of stress-responsive immune cells. Given the extensive processing time to isolate peripheral blood mononuclear cells (PBMCs) from whole blood, the field requires a standardized strategy for suppressing ex vivo gene activation (evGA), to optimally maintain the cells' in vivo transcriptomic profile. Our testing revealed two promising strategies for suppressing evGA: adding a transcription-translation inhibitor (TTi) cocktail, and processing cells cold. Comparative analysis of PBMCs isolated with and without TTis revealed reduced evGA expression and downregulation of pathways involved in cell contact, apoptosis, and inflammatory signaling versus the cells

experiencing evGA. However, TTi treatment impaired responsiveness to lipopolysaccharide stimulation in subsequent in vitro experiments. In contrast, cold isolation maintained experimental flexibility while similarly downregulating evGA. Notably, the addition of TTis during cold isolation offered minimal additional advantages over cold isolation alone. Thus, while both TTis and cold isolation effectively reduced evGA, we found that cold isolation can be a more practical and advantageous approach. Together, our findings highlight the importance of selecting appropriate cell isolation methods to favour accurate biological profiling in studies involving immune cells.

Keyword: *Peripheral blood mononuclear cell, RNA sequencing, Transcriptomic*

Poster Presentations

August 21

Autoantibodies in neurological conditions

#18 Multiple sclerosis plasma IgG aggregates induce complement-dependent neuronal apoptosis

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Grey matter pathology is central to the progression of multiple sclerosis (MS). We discovered that MS plasma immunoglobulin G (IgG) antibodies, mainly IgG1, form large aggregates (>100 nm) which are retained in the flow-through after binding to Protein A. Utilizing an annexin V live-cell apoptosis detection assay, we demonstrated five times higher levels of neuronal apoptosis induced by MS plasma IgG aggregates (n=190, from two cohorts) compared to other neurological diseases (n=116) and healthy donors (n=44). MS IgG aggregates-mediated, complement-dependent neuronal apoptosis was evaluated in multiple model systems including primary human neurons, primary human astrocytes, neuroblastoma SH-SY5Y cells, and newborn mouse brain slices. Immunocytochemistry revealed the co-deposition of IgG, early and late complement activation products (C1q, C3b, and membrane attack complex C5b9), as well as active caspase 3 in treated neuronal cells. Furthermore, we found that MS plasma cytotoxic antibodies are not present in Protein G flow-through, nor in the paired plasma. The neuronal apoptosis can be inhibited by IgG depletion, disruption of IgG aggregates, pan-caspase inhibitor, and is completely abolished by digestion with IgG-cleaving enzyme IdeS. Transmission electron microscopy and nanoparticle tracking analysis revealed the sizes of MS IgG aggregates are greater than 100 nm. Our data support the pathological role of MS IgG antibodies and corroborate their connection to complement activation and axonal damage, suggesting that apoptosis may be a mechanism of neurodegeneration in MS.

Keyword: *immunoglobulins, antibody, IgG1, IgG3, IgG aggregates, Protein A, complement, C1q, C3b, cytotoxicity, apoptosis, neurons.*

#30 Turncoat antibodies unmasked in a model of autoimmune demyelination: "non-pathogenic" anti-MOG IgG can be pathogenic

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Some autoantibodies present in autoimmune diseases have been identified as pathogenic, but most have no known role. This is the case for so-called "non-pathogenic" antibodies to myelin oligodendrocyte glycoprotein (MOG), which bind to MOG fragments in solution, but not to full-length membrane-bound MOG, as determined in a cell-based diagnostic assay [(1, 2)]. Here we report the discovery and characterization of extrafollicular plasmablasts secreting anti-MOG antibodies in mice with experimental autoimmune encephalomyelitis (EAE) induced by immunization with a mutated MOG fragment called bMOG. Single-cell RNA sequencing data show that these cells specialize in producing non-affinity-matured IgG antibodies, composed mainly of IgG1, but also IgG2b and IgG2c. The IgG1 fraction, unlike the other two, would not contain pathogenic anti-MOG antibodies, as predicted by a cell-based assay on bMOG antiserum. Consistently, one of the most abundant anti-MOG IgG1, which we cloned and named C1, is negative in the cell-based assay and does not aggravate EAE when administered to mice immunized with the classic B cell-independent MOG35-55 peptide. However, C1 aggravates EAE by promoting T cell priming in lymph nodes when administered to bMOG-immunized mice. Therefore, this study refutes the dogma that an antibody must bind to an antigen within diseased tissue to be declared pathogenic, as pathogenicity could arise from the capture, elsewhere in the body, of an antigen containing a pathogenic T cell epitope. This pleads for broadening the search criteria for pathogenic antibodies and would explain in part why few pathogenic autoantibodies have been identified to date.

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Keyword: *Autoantibody, demyelinating autoimmune disease, antibody-secreting cell, experimental autoimmune encephalomyelitis, anti-MOG antibody disease*

#33 Antibodies against glutamic acid decarboxylase 65 are locally generated in the cerebrospinal fluid and undergo affinity maturation

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Background: Antibodies against glutamic acid decarboxylase 65 (GAD65) are found in patients with encephalitis, cerebellar ataxia, and stiff-person-syndrome, together referred to as GAD65-Ab-spectrum disorders (GAD65-Ab-SD). So far, little is known about disease pathogenesis[1]. The clinical response of patients to plasma exchange and intravenous immunoglobulin suggests that GAD65-Abs may be involved[2,3]. The role of GAD65-specific B cells as well as their presence in the central nervous system however remain matters of debate.

Objectives: We address the question whether GAD65-antibody producing B cells are present in the cerebrospinal fluid (CSF) by generating monoclonal antibodies (mAbs) from single CSF B cells from 4 GAD65-Ab-SD patients and characterizing their reactivity against GAD65.

Methods: Patient-derived monoclonal antibodies were generated as described[3]. GAD65 reactivity was assessed by cell-based assay, indirect immunofluorescence staining on primate brain tissue and enzyme-linked immunosorbent assay. mAbs were backmutated to their corresponding germline-encoded unmutated common ancestors and re-tested for GAD65 reactivity.

Results: Twelve mAbs were generated, out of which 7 (58%) were GAD65-reactive in at least one detection assay. 4/12 (33%) were positive in all three detection assays. GAD65-reactive mAbs were derived from plasma cells, plasmablasts, and memory B cells. They were mostly of the IgG1 subtype and not clonally related. GAD65 reactivity was abolished after reversion of two GAD65-reactive mAbs to their corresponding germline-encoded unmutated common ancestors.

Conclusions: GAD65-specific B cells are found in the CSF of GAD65-Ab-SD patients and represent a sizable fraction of CSF B cells. The intrathecal anti-GAD65-response is polyclonal and shows evidence of antigen-driven affinity maturation required for GAD65 recognition. Accumulation of GAD65-specific B cells and plasma cells in the CSF may be an important feature of the disease.

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#50 Dissection of complement and Fc-receptor-mediated pathomechanisms of autoantibodies to myelin oligodendrocyte glycoprotein

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Autoantibodies against myelin oligodendrocyte glycoprotein (MOG) have recently been established to define a new disease entity, MOG-antibody-associated disease (MOGAD), which is clinically overlapping with multiple sclerosis. MOG-specific antibodies (Abs) from patients are pathogenic, but the precise effector mechanisms are currently still unknown and no therapy is approved for MOGAD. Here, we determined the contributions of complement and Fc-receptor (FcR)-mediated effects in the pathogenicity of MOG-Abs. Starting from a recombinant anti-MOG (mAb) with human IgG1 Fc, we established MOG-specific mutant mAbs with differential FcR and C1q binding. We then applied selected mutants of this MOG-mAb in two animal models of experimental autoimmune encephalomyelitis. First, we observed that MOG-mAb-induced demyelination was mediated by both complement and FcRs about equally. Second, we found that MOG-Abs enhanced activation of cognate MOG-specific T cells in the central nervous system (CNS), which was dependent on FcR-, but not C1q-binding. The identification of complement-dependent and -independent pathomechanisms of MOG-Abs has implications for therapeutic strategies in MOGAD.

Keyword: *Autoimmunity, Demyelination, Effector mechanisms, Inflammation, Neuroimmunology*

#96 Blood-brain barrier permeability and IgG antibodies infiltration in the brain of a transgenic lupus prone mouse model

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that affects many organs and tissues, including the brain. The term neuropsychiatric lupus erythematosus (NPSLE) includes all the neurological and psychiatric manifestations associated with SLE. People with NPSLE have alterations in the blood-brain barrier (BBB), thrombosis, and auto-antibodies in the brain. In SLE, loss of immune tolerance leads to the production of autoantibodies and the formation of circulating immune complexes (ICs) in blood. ICs bind to Fcgamma receptors (FcgammaRs) that play a major role in inflammation. The FcgammaRIIA receptor is expressed by several immune cells, including platelets and neutrophils in humans. However, FcgammaRIIA is not expressed in mice. The addition of the transgene encoding FcgammaRIIA in a mouse model of SLE accelerates nephritis and platelet activation in vivo. Considering the abundance of platelets and neutrophils in blood, we hypothesized that the activation of these cells by ICs in SLE could impact the integrity of the BBB. In this study, the permeability of the BBB, and the entry of antibodies to the brain were studied in our mouse model of SLE (NZB/NZWF1.FcgammaRIIA^{TGN}) using different imaging techniques (intravital videomicroscopy and postmortem microscopy) by evaluating the passage of fluorescent tracers of different dimensions (40 kDa and 150 kDa) into the brain. While platelet activation, thrombi, and neutrophil interaction with the blood vessel wall was frequent in the brain of NZB/NZWF1.FcgammaRIIA^{TGN} mice, we found a loss of integrity of the BBB that takes place independently of the expression of FcgammaRIIA. Nevertheless, our study revealed that an accumulation of IgG antibodies is higher in the brain of SLE mice expressing the FcgammaRIIA receptor. These findings reveal cellular activation in the brain vasculature as well as the presence of a mechanism responsible for antibody entry, other than the BBB permeability, potentially amplified by the expression of FcgammaRIIA and ICs. This study puts forward our mouse model that can be utilized for the study of the pathogenic roles of ICs in SLE.

Keyword: *Systemic lupus erythematosus, autoantibodies, Blood-brain barrier*

#155 Captopril, an Angiotensin-Converting Enzyme (ACE) Inhibitor, Attenuates Microglial Activation and Improves Social Behavior in a Mouse Model of Autism Spectrum Disorder

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In utero exposure to maternal anti-brain antibodies (IgG) is linked to an increased risk of autism spectrum disorder (ASD) in offspring. Caspr2 protein (Contactin-associated protein-like 2, encoded by the ASD risk gene, CNTNAP2) is a common target of these maternal antibodies. We have developed a mouse model in which dams are immunized with human Caspr2 protein, and hence develop endogenous anti-

Caspr2 IgG. Male offspring, but not female offspring, born to dams harboring anti-Caspr2 IgG throughout gestation exhibit an ASD-like behavioral phenotype featuring repetitive behavior and impaired social behavior. Since Anti-Caspr2 males also show decreased dendritic arborization and reduced dendritic spine density on pyramidal cells in the CA1 region of the hippocampus, we examine the role of microglia in mediating the effect of in utero exposure to anti-Caspr2 ("Anti-Caspr2") or to control ("Control") IgG. We observe microglial activation with altered synaptic pruning in the hippocampus of adult Anti-Caspr2 male mice compared to Control. Anti-Caspr2 male microglia display increased engulfment of VGAT-labeled inhibitory pre-synapses and decreased engulfment of VGLUT2-labeled excitatory pre-synapses, as compared to Control males. Micro-positron emission tomography (MicroPET) scan confirms increased neuroinflammation in the hippocampus, as measured using a [^{11}C]PBR28 radiotracer. We next study whether we can pharmacologically ameliorate the ASD-like phenotype in Anti-Caspr2 mice by suppressing microglial activation using the Angiotensin Converting Enzyme (ACE) inhibitor captopril. Captopril has previously been shown to suppress microglia in mouse models of Alzheimer's disease and neuropsychiatric lupus. Control and Anti-Caspr2 male mice were given daily intraperitoneal injections of ACE inhibitors captopril (BBB-permeable) or enalapril (BBB-impermeable), or the appropriate vehicle, beginning at 3 weeks of age and continuing for 2 weeks. Treatment was maintained during behavioral testing. Captopril was shown to suppress microglial activation and ameliorate the reduction of dendrites and spines in the CA1 region of the hippocampus in Anti-Caspr2 males, compared to Anti-Caspr2 males treated with enalapril or vehicle. Additionally, Anti-Caspr2 males treated with captopril demonstrate longer reciprocal social interaction time in a dyadic play relative to Anti-Caspr2 males treated with vehicle, and similar reciprocal social interaction time to Control males. Single cell RNA-sequencing of hippocampal microglia confirms that in utero exposure to anti-Caspr2 IgG affects microglia at the transcriptional level, and that captopril ameliorates transcriptional changes by downregulating inflammatory genes and upregulating homeostatic genes. Overall, our model of in utero exposure to anti-Caspr2 IgG can be used to examine the role of microglia in ASD and the potential benefits of using microglia-modulating therapeutics.

#184 Exposure in utero to maternal anti-Caspr2 IgG affects parvalbumin interneuron development and hippocampal network activity

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Autism Spectrum Disorder (ASD) is a heterogenous group of neurodevelopmental disorders that is characterized by impairments in social interactions, communication, and the presence of stereotypic behaviors. ASD affects 1 in every 36 children in the United States and is four times more prevalent in boys than in girls. Both genetic and environmental factors converge on deficits in the GABAergic system, suggesting that inhibitory interneurons might be particularly susceptible and contribute to ASD pathophysiology. Several studies, including our own, have demonstrated that 10-20 percent of mothers of a child with ASD harbor brain-reactive antibodies (IgG). One target of these antibodies is Caspr2, a protein involved in neural development and synaptic transmission, and present in up to 40% of mothers with anti-brain antibodies and an

ASD child. We have developed a model in which female mice are immunized with Caspr2 and harbor endogenous polyclonal anti-Caspr2 IgG throughout gestation. Male offspring, but not female offspring, display ASD-like behaviors and exhibit brain abnormalities including a reduction in the GABAergic parvalbumin interneurons (PV) in the cortex and hippocampus. We did not observe changes in the total GABAergic interneuron population suggesting that exposure in utero to anti-Caspr2 IgG selectively affects PV interneurons. The reduction in PV interneurons in Anti-Caspr2 male mice cannot be explained by a reduction in PV progenitor cells or altered migration as number of proliferating progenitor interneurons (phospho-histone H3+) in the medial ganglion eminence (MGE) and the number of migratory MGE interneurons are similar between Control and Anti-Caspr2 mice. Single nucleus transcriptomics of hippocampal GABAergic interneurons revealed significant alterations in gene pathways that are associated with neural transmission and CNS development. Furthermore, immunofluorescent imaging of synaptic protein expression in these mice revealed a reduction of perisomatic inhibitory synapses onto CA1 pyramidal neurons. We next sought to understand how these deficits in PV interneurons might affect neural activity. To this end, we implanted multi-electrodes in Anti-Caspr2 male mice, targeted to the top layers of the CA1 region (strata oriens and pyramidale, corresponding to the location of PVIs), and calculated relative power of the gamma band. Anti-Caspr2 male mice exhibited a significant increase in mid and high gamma oscillations compared to Control mice. Since PV interneurons contribute to hippocampal network synchrony, and dysregulation of these cells is a proposed mechanism underlying ASD; ongoing studies are focused on the trajectory of PV interneuron development and the effect of exposure in utero to anti-Caspr2 IgG on the intrinsic physiology of PV interneurons.

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#190 Do multiple sclerosis autoantibodies target ependymal GlialCAM?

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Background: Multiple Sclerosis (MS) is characterized by demyelinating lesions, including periventricular (PV) lesions, which are near the cerebrospinal fluid (CSF) suggesting that cytotoxic elements in the CSF may drive this damage. The ependyma, the epithelial barrier separating CSF and PV parenchyma, have vital functions such as regulating CSF circulation and metabolic exchange. Ependymal cells are altered in MS and in MS models, including undergoing ependymogliosis, channel dysregulation and cilia damage; but how these cells become damaged and how this is involved in PV lesion formation is

unknown. Oligoclonal bands of immunoglobulin G (IgG) produced by B-cells in CSF are a hallmark feature of MS and the role of B-cell pathogenicity has recently gained interest. A recent study identified IgGs in CSF targeting GlialCAM which is highly expressed by ependymal cells, in addition to astrocytes and oligodendrocytes. Thus, it is highly likely that GlialCAM-targeting antibodies bind these cell types to drive their damage. Given the high incidence of PV lesions in MS, it is particularly critical to understand how IgG-mediated ependyma/PV damage is governed. **Aim:** To evaluate if MS-IgGs bind ependymal/subependymal sites enriched with GlialCAM in situ (1); as well as assess the effects of MS serum and CSF on GlialCAM expression on ependymal cells in vitro (2). **Methods:** Aim 1 - Matched serum and CSF from 10 patients diagnosed with MS were selected from the biobank NeuroBioTec (Hospices Civils de Lyon). Ability of MS IgGs from serum or CSF to bind ventricular/periventricular sites was evaluated by immunofluorescence on rat brain slices co-labeled for GlialCAM and human IgGs. Aim 2 - Purified IgGs from MS serum and CSF were exposed for 24 hours on rat primary cultures of ependymal cells and GlialCAM expression was evaluated by immunocytochemistry. **Results:** Aim 1 - 4/10 MS **sera** showed ependymal/subependymal human IgG labeling at sites enriched with GlialCAM. 7/10 and 4/10 MS **CSF** showed ependymal/subependymal and perivascular/astrocytic endfeet IgG labeling, respectively. Aim 2 - After 24 hours of culturing with purified IgGs from 10 MS **sera**, no modification of GlialCAM expression of rat ependymal cell cultures was observed compared to controls (non-treated cells or cells exposed to IgGs purified from healthy donors). In contrast, 3/10 MS **CSF** induced overexpression of GlialCAM. **Conclusion**: This preliminary study suggests a potential pathogenic role for MS CSF IgGs at the ependyma in a subset of MS patients.

Keyword: *multiple sclerosis, autoantibodies, ependymal cell, GlialCAM*

#196 Pathogenic antibodies in primary progressive multiple sclerosis cerebrospinal fluid

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Primary progressive multiple sclerosis (PPMS) is characterized by progressive worsening of disability without remission from disease onset. We previously reported that pathogenic antibodies in cerebrospinal fluid (CSF) are a distinctive feature of PPMS. Using a novel mouse model, we found that only PPMS CSF or recombinant antibodies (rAbs) derived from B cells in PPMS CSF induced motor disability and hallmark MS pathology in the cervical spinal cord. This was not observed with relapsing-remitting MS (RRMS) or secondary progressive MS (SPMS) CSF or rAbs. A significantly higher incidence of positive human IgG immunostaining was detected on spinal cords of PPMS rAb-injected mice. However, the exact mechanisms by which PPMS rAbs induce pathology have yet to be elucidated. Here, our goal was to investigate whether the Fc region plays an essential role in the pathogenicity of PPMS antibodies.

PPMS CSF was obtained via lumbar puncture and single cells were isolated by FACS. PPMS rAbs and PPMS Fc-silent rAbs were generated after PCR sequencing of heavy and light chains, plasmid expression and purification. LALA-PG mutations were introduced into the Fc-region to disrupt effector functions in Fc-silent rAbs. Mice underwent laminectomies at cervical levels 4 and 5, then 3µl of either: 1) PPMS rAbs, 2) PPMS Fc-silent rAbs, or 3) saline was injected into the cervical subarachnoid space. At 1 day

post injection (DPI), forelimb motor function was assessed and mice were immediately perfused for histological analyses.

Mice injected with PPMS rAbs developed significant forelimb motor disability by 1 DPI. However, normal forelimb function was maintained in mice injected with PPMS Fc-silent rAbs. Preliminary data show that PPMS Fc-silent rAb-injected mice exhibit attenuated pathology in the cervical spinal cord, unlike PPMS rAb-injected mice that develop demyelinated lesions, reactive astrogliosis and axonal damage. Human IgG immunostaining also appeared to be reduced in PPMS Fc-silent rAb-injected mice compared to PPMS rAb-injected mice.

Overall, our study shows that the pathogenic capacity of PPMS rAbs is attenuated when the Fc region is silenced, suggesting that the Fc region of PPMS antibodies plays a critical role in its pathogenicity. Future studies will explore the contribution of specific effector functions mediated by the Fc-region to the development of motor disability and pathology in PPMS.

Keyword: *primary progressive multiple sclerosis, pathogenic antibodies, cerebrospinal fluid*

#218 Laboratory diagnostic strategies for the identification of antibodies against neuronal synaptic antigens in autoimmune encephalitis

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Introduction

The detection of antibodies against neuronal synaptic proteins (NSAbs) is a crucial step in autoimmune encephalitis (AE) diagnosis, but different laboratory assays are available[1,2,3]. We aimed to compare the performance of commercial and in-house assays for NSAbs detection and to define the relevance of uncharacterized NSAbs (unc-NSAbs) identified only on in-house rat brain tissue based assays (TBA).

Methods

We tested for NSAbs 1112 samples sent for suspect AE using commercial cell-based assay (C-CBA) and TBA[4]. All positive patients on TBA and/or C-CBA were additionally tested using in-house CBAs (ih-CBA). Unc-NSAbs were tested on live rat neuronal cultures (LNC). We analysed the predictive relevance of TBA staining pattern using multivariate analysis.

Results

We included 778/1112 patients and 79/778 (10.2%) had AE (Fig.1). All C-CBA+/TBA+ patients diagnosed with AE, and none of the C-CBA+/ihTBA-. The TBA showed the highest negative Likelihood Ratio (Table 1). Forty-

three/44 of unc-Nabs patients were not diagnosed with AE, and 3 of them were positive using LNC. Both CSF and serum were analyzed in 442/778 patients. Serum-only positivity in any assay occurred only in non-AE patients. The only TBA pattern predictor for AE diagnosis was a higher intensity of CSF staining ($p<0.001$).

Conclusion

Commercial kits for NSAbs detection have lower accuracy compared to in-house assays. The best diagnostic strategy should combine TBA as a screening test, followed by ih-CBA, preferentially using both serum and CSF. In our study, Unc-NAbs were not clinically relevant, and LNC do not seem to increase the diagnostic accuracy.

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Keyword: *Autoimmune encephalitis, Laboratory diagnostics, Antibodies against neuronal synaptic proteins*

#234 A highly sensitive split luciferase assay for the detection of functional acetylcholine receptor autoantibodies

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Autoantibodies to acetylcholine receptors (AChRs) are found in myasthenia gravis (MG) and autoimmune autonomic ganglionopathy (AAG). Functional AChR autoantibodies exert their effects via immunomodulation, with receptor cross-linking and internalisation following antibody binding. However, most commercial immunoassays for the diagnosis of MG and AAG do not detect the immunomodulating effects of AChR binding antibodies, limiting their diagnostic capabilities.

We developed novel, high sensitivity, bioluminescent live cell immunoassays for the detection of functional AChR antibodies. Patient sera were incubated overnight with either IMR-32 or RD cells, cell lines which express conformational surface ganglionic and muscle AChR respectively. Remaining surface AChR was then quantified from a bioluminescent signal generated using a split luciferase detection system. The degree of AChR receptor immunomodulation following serum incubation was calculated from comparison to reference healthy sera.

The assays were able to detect immunomodulating antibodies in patients with MG and AAG, with high sensitivity and specificity, compared to disease and healthy controls without AChR antibody mediated disease. Autoantibodies remained detectable in up to 1:10 000 dilutions of antibody positive patient serum demonstrating the assays' high analytical sensitivity. Results from our assays had high levels of agreement with those obtained from a previously validated flow cytometric method.
The split luciferase detection system in our assays permits rapid signal acquisition, has a wide dynamic range and reduces the cumbersome wash steps required in other immunoassays. Therefore, our assays offer a new ultra-high throughput method for functional AChR autoantibody detection with high sensitivity, and may overcome the limitations of current commercial diagnostic methods. Extensive assessment and validation with further patient and control sera will be performed.

Keyword: *myasthenia gravis, acetylcholine receptor, autoantibodies, autoimmune autonomic ganglionopathy*

#246 Elevated EBNA1 Antibodies Found in Multiple Sclerosis Patients Compared to Healthy Controls

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Hypothesis: Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system. There is an increased intrathecal antibody synthesis in MS patients, which could be caused by increased viral activity. The role of Epstein-Barr virus in MS is a hot topic. Could quantification of antibodies to Epstein-Barr Virus antigen EBNA1-full length (EBNA1-FL) be used to discriminate between MS patients and healthy controls?

Methods: Specific IgG concentrations against EBNA1-FL in paired cerebrospinal fluid (CSF) and serum samples were examined by direct enzyme-linked immunosorbent assays. Samples were from 78 patients with MS from Rigshospitalet and 15 healthy controls.

Results: Significantly elevated responses were recorded in patients with MS compared to healthy controls. CSF analyses were not more sensitive than serum analyses. Higher sensitivity or specificity was not obtained when normalizing to total albumin concentration or total IgG concentration.

Discussion: Antibody levels against EBNA1 can be used to discriminate between healthy controls and MS patients depending on the type of antigen and interindividual variances in antibody productions.

Keyword: *Multiple Sclerosis, EBNA antibody Elisa*

#290 Clinical features of autoimmune nodopathy with Caspr1 antibodies in South Korea

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Background

Autoimmune nodopathy is an immune mediated neuropathy, revealed the identification of several adhesion molecules located in the node or paranode region as antigens for autoantibodies. Contactin-associated protein 1 (Caspr1) is a one of these autoantibodies but prevalence and clinical characteristics of patients with positive anti Caspr1 antibodies are still largely unknown. We aimed to investigate the clinical features of Korean patients with Caspr1 antibody positive autoimmune nodopathy.

Method

Sera from 98 patients fulfilling diagnostic criteria of chronic inflammatory demyelinating polyneuropathy (CIDP) were screened for Caspr1 using enzyme-linked immunosorbent assay (ELISA). The Caspr1 positive sera were additionally tested for neurofascin 155, anti-neurofascin 186, CNTN1 antibody and immunoglobulin G (IgG) subclasses for Caspr1 antibodies. Clinical features were reviewed retrospectively.

Result

Among the 91 patients, four (4.4%) was positive for Caspr1 antibody. In the four patients with Caspr1 antibody, two were also positive for CNTN1 antibody in ELISA, but none of them were detected for anti-neurofascin 155 and anti-neurofascin 186 antibodies. In all the four patients, IgG4 was the predominant isotype of Caspr1 antibody. CSF protein levels were elevated in three patients without pleocytosis. Mean age at onset is 65.3 years. Two patients were males the other two patients were females. All four patients presented with progressive paresthesia in distal limbs. Three patients had sensory ataxia without evident motor weakness, while one patient had a significant motor weakness (MRC grade II ~ III). Among the three patients with sensory ataxia, two had severe neuropathic pain in both hands and toes. None of the four patients had any cranial nerve involvement. There were no other respiratory or autonomic symptoms observed. Two patients treated with intravenous immunoglobulin (IVIg), showed partially improvement. One patient was treated with plasma exchange and showed a good response.

Conclusion

In this study, anti-Caspr1 antibody was found in approximately 4% of Korean patients fulfilling CIDP diagnostic criteria. Painful paresthesia, sensory ataxia, and CSF protein elevation were characteristic clinical features in the autoimmune nodopathy patient with Caspr1 antibody. Large-scale further studies are needed to clarify the clinical features of Capr1 antibody.

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relevance of IgG isotype. Neurol Neuroimmunol Neuroinflamm 2020;7.

Keyword: *Peripheral neuropathy, Autoimmune diseases, Nodopathy, Autoantibody, Caspr1*

#361 Autoimmune Encephalitis Against R-Type Calcium Channel, a New Type of Encephalitis Proposal and Case Report

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BACKGROUND AND AIMS

R-type voltage gated calcium channel Cav2.3 is encoded by the CACNA1E gene. It is located on chromosome 1q25.3. It is highly expressed in the cortex, hippocampus, striatum, amygdala, interpeduncular nucleus and the cerebellar cortex anterior lobe. Mutations in CACNA1E gene are considered pathogenic and strongly associated with developmental and epileptic encephalopathy 69 (DEE69), a severe autosomal dominant pediatric disorder characterized by early-onset refractory seizures, abundant epileptiform activity on EEG and developmental impairment or regression. It's treatment is based on antiepileptics such as topiramate and lamotrigine. DEE69 has been exceptionally reported in adults. The aim is to report a new type of Autoimmune Encephalitis (AE) against the R-type Calcium Channel.

METHODS

A 40 year old hispanic female presented to clinic with symptoms resembling viral meningitis, intracranial hypertension, pseudobulbar affect, anxiety, disorientation, psychosis with hallucinations, hyperkinesia and dissociative amnesia. Physical examination showed ecolalia with neologisms, orofacial dyskinesias, dyscalculia, prosopagnosia, hyperreflexia, bilateral Hoffman's, bilateral Marinescu and gait ataxia. MoCA test was 15/30. MRI showed left frontal cortical and subcortical hyperintense lesions, bilateral parieto-occipital, lenticular and thalamic lesions. EEG showed frontal extreme delta brush pattern with superimposed supramaximal beta pattern. Whole-exome sequencing (WES) evidenced CACNA-1E gene mutation.

RESULTS

Early treatment was initiated with methylprednisolone (MTP) and intravenous immunoglobulin (IVIG) with fairly good and quick response. At 4 weeks patient relapsed with altered mental status, behavior changes, hypersexuality and movement disorders. Administration of a second MTP cycle including rituximab exhibited a rapid and favorable response, especially to corticosteroid therapy. After 1 month the patient had completely recovered. MOCA reached 29/30.

CONCLUSIONS

We propose a new type of AE against the R-type Calcium Channel. Due to the rarity of CACNA-1E gene mutation and the limited reported cases, further research is necessary to understand the underlying mechanisms and pathophysiology of this gene.

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Keyword: *CACNA1E, ENCEPHALITIS, R-TYPE VGCC*

#379 Developing aquaporin-4 autoreactive B cells favor extracellular loop A epitopes

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Autoantibodies targeting the extracellular domains of the aquaporin-4 (AQP4-IgG) water channel play a pathogenic role in neuromyelitis optica spectrum disorders (NMOSD). Molecular and clinical data support that at clinical symptom onset, the repertoire of AQP4-autoreactive B cells has undergone significant maturation, however few studies have investigated the underlying mechanisms driving this process. Yet, it is known that specific epitopes significantly influence complement activation and consequent pathogenic potential of patient-derived AQP4 monoclonal antibodies. Given this intimate link between epitopes and pathogenicity, we hypothesized that B cell receptor epitope specificities influence selection of early AQP4-reactive B cell populations. To test this hypothesis, individual AQP4-reactive naïve and memory B cell receptors were isolated from three patients with NMOSD. After FACs and cell cultures, heavy and light immunoglobulin chains from positive wells were PCR amplified and cloned into vectors, to generate AQP4-specific monoclonal antibodies (AQP4-mAbs, n=25 total). These were exploited as molecularly precise tools to investigate the B cell receptor – AQP4 interaction. Epitope specificity was mapped by quantifying AQP4-mAb binding to HEK cells expressing wildtype M23-AQP4 compared to M23-AQP4 with single extracellular amino acid point mutations. The extracellular epitopes for all AQP4-mAbs utilized a diverse combination of amino acids spanning multiple extracellular loops. No single mutation was distinct when comparing developing vs memory B cell populations. Binding for the overwhelming majority of AQP4-mAbs isolated from developing B cell populations was dependent on loop A amino acids (21/22 mAbs), a much higher percentage than that observed within the memory population and published reports quantifying AQP4-IgG titers within the periphery and CNS. Other functional metrics including AQP4-mAb binding affinity, CDR3 length or charge, or complement-dependent cytotoxicity

EC50 were not enriched within the memory population. The results suggest that a diverse yet restricted set of epitopes are over-represented in mature B cell populations, warranting further investigations into how these particular epitopes may modulate or govern B cell receptor maturity.

#387 Acetylcholine receptor antibodies isoform specificity as potential prognostic biomarker in myasthenia gravis

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INTRODUCTION

Myasthenia gravis (MG) is the most common disorder of neuromuscular junction (NMJ), caused by autoantibodies (auto-Abs) impairing the neuromuscular transmission, leading to voluntary muscle weakness. Most patients present auto-Abs directed against the acetylcholine receptor (AChR), displaying a specificity against both the adult (A) and foetal (F) AChR isoforms. Although the detection of AChR-Abs is important in confirming the MG diagnosis, their titre does not correlate with the disease outcome, and, to date, there are no reliable predictive biomarkers of the MG clinical outcome. To fill this gap, we assessed whether the preferential antibody reactivity for the A- and F-AChR isoform and the AChR-IgG subclass switching during disease course could correlate with the MG clinical outcome.

METHODS

We conducted a cross-sectional/longitudinal study on 174 patients with a confirmed diagnosis of MG and positive for AChR-Abs by RIA from two referral MG centers. The antibody reactivity against A-AChR vs F-AChR isoforms (A/F ratio level) and AChR-IgG subclasses modifications were assessed by live cell based assay on flow cytometry. The clinical outcome was measured by using the "patient acceptable symptom state" (PASS question) and the Post-intervention status (PIS).

RESULTS

We found a significantly lower A/F ratio level in patients who replied "yes" to the PASS question compared to those who replied "no" (p=0.039, Mann-Whitney test) showing that patients with good clinical outcome (e.g., PASS=yes) have Abs mostly binding to the foetal isoform of AChR. This finding was confirmed when we compared the A/F ratio level in patients in minimal manifestation or better ("MM or better") vs symptomatic ones (p=0.001, Mann-Whitney test). The A/F ratio level was particularly low in complete stable remission (CSR) patients vs MM (p=0.04, Mann-Whitney test). No significant difference in the A/F ratio was found when comparing patients with ocular vs generalized MG, patients with thymoma vs non thymoma, and naïve to immunotherapy or under treatment. The AChR-IgG subclass profile was similar for A-AChR and F-AChR-Abs and no significant correlation were found with the clinical outcome both by using PASS and PIS outcome values. However, over time, we identified a switch from a predominant subclass to another, suggesting dynamic changes in the antibody-producing B cell subsets during the disease course.

CONCLUSION

The A/F AChR IgG ratio could represent a promising biological predictive marker of AChR-MG disease outcome, while AChR-IgG subclasses changes did not show any potential to predict MG outcome, but could represent an indirect way to monitor B cell subsets changes longitudinally. Large multicentric prospective studies are needed to confirm our results.

Keyword: *myasthenia gravis, acetylcholine receptor, autoantibodies, biomarker*

Immuno-neuro-oncology

#144 Effect of Cisplatin on Microglia and Kidney-Resident Macrophages: An Ultrastructure Study

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Background: Cisplatin, an antineoplastic drug, is commonly used to treat solid tumours in a variety of cancers. However, patients undergoing cisplatin treatment often report symptoms of both physiological and cognitive fatigue. Cisplatin and its metabolites are primarily filtered and removed by the kidney where they accumulate and induce their cytotoxic effects, but trace amounts have also been found in the brain [1]. As the innate immune cells native to the kidney and brain, respectively, have important roles critical for removing waste, repairing tissue damage, and dealing with immune challenges, we hypothesized that cisplatin induces a cellular stress response detectible at the ultrastructural level.

Methods: Adult male mice were exposed to one or two 5-day cycles of cisplatin treatment (2.83 mg/kg/day, n= 4 per treatment) of which the effects were measured by decreased wheel running and body weight loss. Chip mapping scanning electron microscopy was used to investigate the ultrastructural impact of cisplatin on kidney-resident macrophages in the renal cortex—the region largely responsible for cisplatin filtration—and microglia in the periventricular striatum—where high amounts of ion transporters used by cisplatin are expressed.

Results: Two cycles of cisplatin treatment tended to increase endoplasmic reticulum stress in the innate immune cells of the renal cortex and brain's striatum. Both one and two cycles of cisplatin treatment significantly increased the presence of mature lysosomal bodies in kidney-resident macrophages as well as the number of contacts with kidney tubular cells. Two cycles of cisplatin treatment also increased parenchymal microglial contacts with synaptic elements—supporting previous research describing the role of microglia in chemotherapy-associated cognitive impairment [2]. Overall, both kidney-resident macrophages and microglia expressed moderately increased markers of cellular stress compared to cells from control animals.

Implications: These findings demonstrate the profound ability of macrophages and microglia to respond to environmental stressors and have implications for patients undergoing and recovering from cisplatin treatment. The development of interventions to reduce microglia reactivity to cisplatin treatment might alleviate cognitive side effects.

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Keyword: *Microglia, Cisplatin, Inflammation, Kidney, Electron Microscopy*

#281 Concurrent cancer near the onset of MOG antibody associated disease

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Background: Myelin oligodendrocyte glycoprotein antibody associated disease (MOGAD) has been identified as a distinct inflammatory demyelinating disease of the central nervous system. Several studies have suggested an association between other autoimmune diseases and cancer. Although the MOG antibody is not thought to be closely associated with paraneoplastic neurological syndromes, the incidence of cancer near the onset of MOGAD has not previously been investigated.

Objectives and Aims: The aim of this study is to assess the incidence of cancer near the onset of MOGAD in a worldwide cohort. We also discuss cases of MOGAD that presented with a concurrent cancer.

Methods: Among 622 patients with MOGAD in this worldwide cohort, 93 patients were excluded due to short follow-up period (< 12 months). A total of 529 patients were included from 9 tertiary referral hospitals in South Korea and Mayo Clinic in US from August 2012 to April 2023. Concurrent cancer was defined as the cancer identified within 12 months of the diagnosis of MOGAD. The standardized incidence ratio (SIR) was calculated based on national cancer incidence data of each country. Subgroup analysis was done among adult (aged 18 years or over) onset and elderly (aged 50 years or over) onset populations.

Results: The median ages at onset of MOGAD diagnosis were 27.3 (interquartile range, IQR; 12.0 - 49.1) in the Korean cohort and 31.0 (IQR; 13.7 – 47.6) in the Mayo cohort. Females constituted 52% of the Korean cohort and 60% of the Mayo cohort, and median disease durations were 3.9 years (IQR; 2.3 – 7.9) and 4.7 years (IQR; 2.2 – 8.9), respectively. The observed incidence rate of concurrent cancer within 12 month of MOGAD diagnosis was 2.37 per 100 person-year (py) in the Korean cohort and 0.70 per 100 py in the Mayo cohort. While the expected incidence rates based on population data were 0.36 per 100 py in Korea and 0.27 per 100 py in the Mayo. The SIR of concurrent malignant tumor is significantly high in the Korean cohort (SIR = 6.62, 95% CI 3.42 – 11.56, $p < 0.001$), especially higher in adult onset MOGAD patients (SIR = 7.87, 95% CI 4.06 – 13.75, $p < 0.001$). The SIR of concurrent malignant tumor in the Mayo cohort was also high at 2.56 although statistically insignificant (95% CI 0.69 – 6.56, $p = 0.07$). In the total cohort of MOGAD, the SIR was significantly high in the

total group (SIR = 4.74, 95% CI 2.71 – 7.70, p < 0.001), adult onset group (SIR = 5.25, 95% CI 3.00 – 8.52, p < 0.001), and elderly onset group (SIR = 4.97, 95% CI 2.57 – 8.68, p < 0.001). Of the 12 patients with MOGAD with concurrent cancer, 4 had adenocarcinoma (colon cancer 2, pancreatic cancer 2). Other cancer types included breast cancer (n=2), T-cell lymphoma (cutaneous 1, intestinal 1), chronic myeloid leukemia (n=1), multiple myeloma (n=1), small cell lung cancer (n=1), stomach cancer (Signet ring cell carcinoma, n=1), renal cell carcinoma (n=1), papillary thyroid cancer (n=1), mesothelioma (n=1), and metastatic melanoma (n=1).

Conclusions: Concurrent cancer may be seen in patients with MOGAD at a higher rate than the general population, especially in adult onset patients. Further prospective large cohort studies will be needed in this field to confirm these findings.

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Keyword: *MOGAD, Standardized incidence ratio, Myelin oligodendrocyte glycoprotein, Cancer, Concurrent*

#378 The Good, Bad and Ugly Side of Cancer Immunotherapy: Neuroinflammation and Cognitive Dysfunction Following Immune Checkpoint Inhibition

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Each year, approximately 2 million Americans will be diagnosed with cancer. As cancer therapies have become more effective, leading to longer life expectancies, there has been a massive influx of cancer survivors suffering from unintended, debilitating side effects, including cancer therapy-related cognitive impairments (CRCI). Our past radiation- and chemo-therapy studies suggest that elevated peripheral- and neuro-inflammation plays a detrimental role in neural damage, including CRCI. While the neurobiological mechanisms and mitigation strategies for CRCI have been determined, a comprehensive understanding of the mechanism of immune checkpoint inhibition (ICI)-induced brain injury and cognitive dysfunction remains to be established. ICI allows the immune system to recognize cancer cell antigens to trigger T cell-mediated immune response to eliminate cancer. In doing so, T cells and other tumor-infiltrating immune cells release pro-inflammatory cytokines (TNFα, INF_{γ}, IL1β), leading to the systemic immune response. These pro-inflammatory cytokines may also cross the bloodbrain barrier and trigger neuroinflammation in the brain leading to neuronal and synaptic damage. Our study has characterized the neuro-inflammatory response following ICI treatment for melanoma as well as the extent of cognitive impairments. C57Bl6 mice underwent melanoma tumor induction and were treated with a combinatorial ICI treatment (anti-PD1 and anti-CTLA4 antibodies) for 3-weeks. A month post-ICI treatment, mice underwent behavior testing, including anxiety (open field test, OFT; elevated plus maze, EPM; light-dark box, LDB), learning and memory (novel place/object recognition; NPR, NOR), and memory consolidation (fear extinction, FE) tasks. ICI treatment significantly reduced melanoma progression. ICI treatment in

the mice with or without tumors did not affect the performance on OFT, EPM, or NOR tasks. However, we found a significant decrease in performance in LDB, hippocampal-dependent NPR, and FE (P's<0.02) tasks. These data indicate the hippocampal-selective neurocognitive impact of ICI treatment. IHC assessment of CD68-IBA1 positive microglia showed elevated microglial activation following ICI treatment. We also found reduced synaptic integrity and neurogenesis in the ICI-treated brains. Our data present a mammalian model of cancer immunotherapy-mediated cognitive impairments and support our hypothesis that long-term neuroinflammation is a major contributing factor in CRCI.

Keyword: *oncology, immunotherapy, melanoma, immune checkpoint inhibition, T cells*

#392 Study of neuroimmune gamma-aminobutyric acid and glutamate signaling pathways in glioblastoma

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Introduction. Gliomas make up the majority of malignant primary brain tumors, and cannot usually be treated curatively. It therefore becomes necessary to identify which mechanisms are responsible for tumor survival and proliferation in order to identify new therapeutic targets. There are several lines of evidence suggesting that amino acid neurotransmitters gamma-aminobutyric acid (GABA) and glutamate are involved in gliomagenesis. However, the mechanisms, and the elements of the tumor microenvironment (TME) responsible are poorly defined.

Hypothesis. We hypothesize that by modulating GABA or glutamate genes, we may be able to control tumoral aggressivity through regulation of neuroimmune cell pathways, which represent a significant part of the TME. In this study, we identify vulnerable biomarker genes for glioblastoma (stage IV glioma) using bioinformatic, immunohistochemical (IHC), and cellular techniques.

Methodology and results. A GABA-treated U87 glioblastoma (GBM) cell line was first bulk RNA sequenced, and enrichment analysis was performed. Data suggests regulation of many cancer pathways, such as survival and metabolism. Single-cell RNA sequencing data of 9 resected IDH-wildtype GBM further reveals high cellular heterogeneity within the TME. Using a novel algorithmic cell-type identification approach, GBM cells, along with neuroimmune populations displaying both pro- and anti-inflammatory characteristics were identified. The environment notably includes NK cells, neutrophils, microglia, M1 and M2 microglia-derived tumor associated macrophages, different subtypes of T cells, reactive astrocytes, and dendritic cells. Specific potential GABA, glutamate, and calcium neurotransmitter-associated biomarkers identified by previous studies in the laboratory were found to be differentially expressed between cell-group clusters within the TME. Further enrichment analysis aims to identify cell types, and genes associated with protumoral activity. A cohort comprising 50 resected GBM samples was utilized to associate those new genes, as well as previous markers C5AR1, VGAT, GAD-1, and GABA-B to histologic characteristics, such as necrosis, angiogenesis, and infiltration. Additional IHC alignment between our markers, and clinical markers such as MIB-1 (for

proliferation) was performed. GABA was significantly overexpressed in MIB-1 rich zones in GBM.

Conclusion. This study seeks to introduce GABA- and glutamate-associated biomarkers as potential therapeutic vulnerabilities in glioblastoma.

Inflammation in neurodegenerative diseases

#4 Inhibiting nighttime melatonin and boosting cortisol increase patrolling monocytes, phagocytosis, and myelination in a murine model of multiple sclerosis

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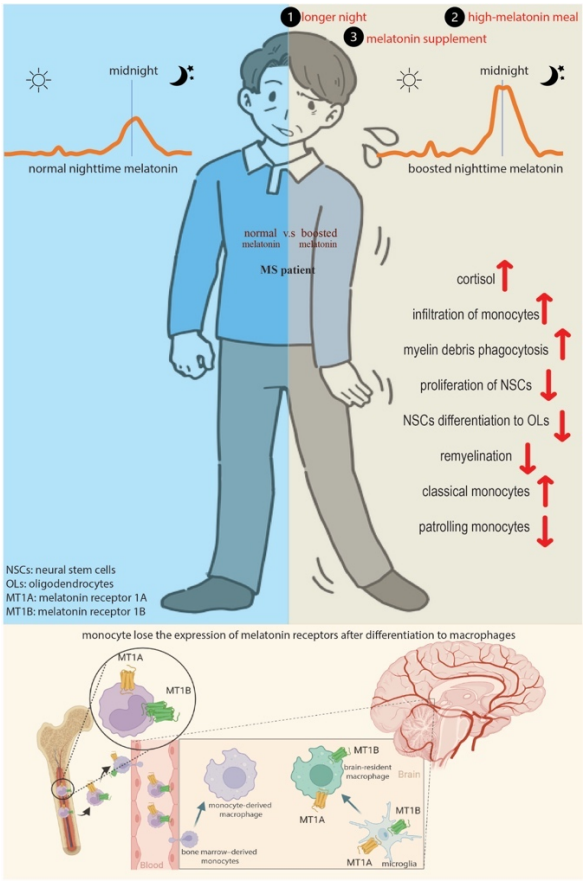

Background: Conflicting results are reported concerning melatonin synthesis in multiple sclerosis (MS) likely due to variabilities between patients' lifestyles, not considered when supplementing melatonin. Here, we aimed to investigate the melatonin/cortisol alterations by manipulation of circadian light:night cycle and exogenous melatonin.

Methods: The cuprizone model was used which mimics several characteristics of progressive MS. Synthesis of both circadian melatonin and cortisol were manipulated by our novel approach of light-

darkness. In addition, since melatonin acts through its receptors, we used the receptor agonist, melatonin, and antagonist, luzindole, while we identified the receptor expression in our model. Furthermore, chimeric mice were used to discriminate bone marrow (BM)-derived macrophages from brain-resident macrophages and also for examination of infiltration. We further studied neural stem/progenitor cells (NSPCs) proliferation, differentiation to oligodendrocyte precursor cells (OPCs), and their recruitment to the site of demyelination and maturation to oligodendrocytes (OLs). Furthermore, monocyte subtypes and macrophages were also evaluated.

Results: We identified melatonin receptors in: OLs of the corpus callosum, where demyelination happens; subventricular zone, where NSPCs are located, and choroid plexus, where it functions as a blood-cerebrospinal fluid barrier. Moreover, residential macrophages were found to express melatonin receptors, whereas BM derived macrophages lose this expression in the demyelinated brain. Next, we showed that cuprizone-fed mice tend to increase melatonin levels. While we employed different approaches to alter circadian rhythm of melatonin and cortisol, only the constant light approach increased cortisol level, NSPC's proliferation and differentiation to OPCs, their maturation to OLs and recruitment to the site of demyelination, the number of patrolling monocytes, and phagocytosis. In contrast, constant darkness and exogenous melatonin exacerbated all these events and amplified infiltration of monocytes.

Conclusions: Melatonin should not be considered as a universal remedy, as currently claimed. Boosting melatonin levels, to a value higher than its normal level, in autoimmune diseases including MS amplifies the immune system function and exacerbate the diseases. Our data emphasize the importance of monitoring melatonin/cortisol oscillation in each MS patient, to avoid melatonin's overdose.

Keyword: *multiple sclerosis, circadian rhythm, melatonin, monocyte, cortisol*

#5 Increasing Neuroimmunological Vulnerability Triggered by COVID-19: A case report of Pediatric Onset Multiple Sclerosis (POMS)

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Introduction : We report a case of pediatric-onset multiple sclerosis triggered by COVID-19 and herpes simplex virus, and the phenomenon accompanied by sequential viral infections may increase the neuroimmunological vulnerability in a healthy individual.

Case : A 12-year-old unvaccinated boy with disoriented mental status and headache visited our emergency department. In the initial neurologic examination, he showed a swaying gait appearance and tilted walking on the left side. His previous medical history showed normal development but had been infected with COVID-19 two weeks ago. In his initial serologic results, procalcitonin, d-dimer and interleukin-6 were mildly elevated; the other serologic markers were within normal range.

On the initial brain MRI, hyperintense lesions were involved in the medial temporal lobe, right hippocampus, right frontal base, right frontal lobe medial cortex and basal ganglia and insula. His cerebrospinal analysis showed prominent leukocytosis consisting of dominant lymphocytes and highly elevated cytokine levels, and herpes

simplex virus type 1 was detected in the polymerase chain reaction (PCR) based film array. The initial oligoclonal band was negative. Intravenous acyclovir and methylprednisolone treatment was executed immediately, and he recovered orientation and relieved other symptoms.

Two months later, he complained of dyspepsia and general weakness and visited outpatient clinics. His second brain MRI revealed newly diffuse multiple high-signal lesions in the right peri-insular sulcus, anterior longitudinal cerebral fissure, medial frontal gyrus, and left frontal base. In the second cerebrospinal analysis, the oligoclonal band was positive. The second time methylprednisolone and immunoglobulin were injected, he had tapered down as oral prednisolone for six months.

Conclusion : Sequential virus infections may increase the vulnerability of neuroimmunological diseases such as multiple sclerosis, acute disseminated encephalomyelitis, and guillain-barre syndrome. We suggested that COVID-19 may induce an unbalanced immunologic condition in a healthy child without any underlying susceptibility.

Keyword: *COVID-19, HSV type1, pediatric, encephalitis, multiple sclerosis*

#6 Astrocyte activation with dominant deleterious A1 phenotype in late-stage mice with Spinal Muscular Atrophy.

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Introduction: Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by genetic defect of survival motor neuron (SMN) protein. The widely used rodent model for studying SMA type II is called SMNΔ7.

Methods: For the present study triple transgenic FVB.Cg $Grm7^{Tg(SMN2)89Ahmb}$ $Smn1^{tm1Msd}$ Tg(SMN2*delta7)4299Ahmb/J mice were euthanized postnatally on day 13. Spinal cord (L1-L5) and brain was harvested. Paraffin sections were used for immunofluorescence of astrocytes with GFAP, S100A10 and C3. Frozen tissue was used for western blot for plastin 3 (PLS3) and real-time PCR analyses for Nerve Growth Factor (NGF), Brain Derived Neurotrophic Factor (BDNF) and Vascular Endothelial Growth Factor (VEGF).

Results: Increased activation of two astrocyte markers was detected on $Smn1^{-/-}$ $SMN2^{+/+}$ $SMNΔ7^{+/+}$ (SMNΔ7) mice in contrast to $Smn1^{+/+}$ $SMN2^{+/+}$ $SMNΔ7^{+/+}$ (healthy control / HC). In spinal cord GFAP+ cells were increased in SMNΔ7 mice in contrast to HC (233±9.29 vs. 43.5±5.75, $p<0.0001$) and the same trend was observed for C3+ A1 astrocytes (20.4±2.05 vs. 15.2±3.6, $p<0.0001$), respectively. S100A10+ cells had the same expression level on both SMNΔ7 mice and HC with no statistically significant difference. Actin-binding PLS3 protein levels were decreased in SMNΔ7 mice (0.8±0.06) in contrast to HC mice (1.0±0.0) $p<0.05$ in spinal cord and in brain region (0.72±0.04 vs 1.0±0.0) with $p<0.0001$, respectively. Moreover, SMNΔ7 mice exhibited reduced levels of neurotropic factors such as NGF (0.21±0.34 vs. 1.0±0.0, $p<0.05$) and BDNF (0.09±0.08 vs. 1.0±0.0, $p<0.0001$) in contrast to HC mice in spinal cord. On the contrary VEGF-A levels were increased in SMNΔ7 mice (3.12±0.27) in contrast to HC (1.0±0.0), $p<0.05$. In brain, NGF levels were decreased in SMNΔ7 mice in contrast to HC (0.8±0.04 vs 1.0±0.0) $p<0.05$ and so were for BDNF (0.07±0.24 1.0±0.0) $p<0.05$. VEGF-A

expression levels did not differ between the two experimental groups.

Conclusions: Our preliminary results indicate that SMNΔ7 late-stage mice exhibit increased astrocyte activation with dominant deleterious A1 phenotype, reduced neurotropic support and decreased levels of actin-binding proteins that attribute to cytoskeletal remodeling and axonal transport.

Disclosures: The present study is funded by Biogen in the frame of Investigator Initiated Trial (IIT) [GR-SMG-11658] entitled "Non-neuronal cellular elements of the Central Nervous System and structural biomarkers in an experimental model of Spinal Muscular Atrophy".

Keyword: *Spinal Muscular Atrophy, Astrocytes, actin-binding proteins, glial cells, SMNΔ7 mouse model*

#10 Myelin insulation as a risk factor for axonal degeneration in autoimmune demyelinating disease

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Axonal degeneration determines the clinical outcome of multiple sclerosis (MS), and is thought to result from exposure of denuded axons to immune-mediated damage. We challenge this view after finding in MS and its mouse models that myelin itself increases the risk of axons to degenerate under inflammatory conditions. We propose a model for demyelinating diseases in which for axons that remain myelinated, and thus shielded from the extracellular milieu, dependence from oligodendroglial support turns fatal in an autoimmune disease environment.

Keyword: *multiple sclerosis, EAE, myelin, axonal damage*

#15 Histopathological heterogeneity of microglia in amyotrophic lateral sclerosis

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[Background]
Amyotrophic lateral sclerosis (ALS), an intractable neurodegenerative disease of the central nervous system, is pathologically characterized by motor neuron loss. Although the etiology of ALS remains unknown, its pathophysiology is heterogeneous. A series of recent studies have reported detection of disease-associated microglia (DAM) in lesions of various neurodegenerative diseases. However, few reports have described DAM in sporadic ALS. The role of microglia including DAM in sporadic ALS remains unclear.

[Methods]
We analyzed data obtained from 28 patients with ALS. Autopsy samples of cervical and lumbar lesions were obtained from each patient, and we performed immunohistochemical analysis using anti-TMEM119, anti-Iba1, anti-CX3CR1, anti-CD68, and anti-thrombomodulin antibodies to investigate the role of microglia in the pathogenesis of ALS. Moreover, we investigated the association between microglia surface markers and clinical data including the degree of lower motor neuron loss, clinical symptoms, and disease duration.

[Results]
Spinal cord lesions in ALS can be pathologically categorized into the DAM-dominant and DAM-independent inflammatory neurodegeneration subgroups based on the type of microglia involved. Moreover, expression of CD68 (a microglial activation marker) and endothelial activation were also observed in the TMEM119+ microglia-positive group, which highlights the role of inflammation in ALS. We observed no intergroup differences in sex, riluzole administration for ALS, use of antibiotics, anti-inflammatory drug use, and autopsy findings of histopathologically confirmed chronic pneumonia. We observed no difference between the TMEM119+ and TMEM119-microglia groups with regard to the percentage of patients with unexplained elevation of serum C-reactive protein levels.

[Discussion]
DAM suppresses TMEM119 expression; therefore, TMEM119+ microglia-positivity may indicate DAM-independent inflammatory neurodegeneration.

[Conclusion]
The results of this study may explain the mechanism underlying suppression of disease progression observed in only a few patients in some clinical trials of anti-inflammatory drugs administered for ALS.

Keyword: *ALS, microglia, DAM*

#20 Investigation of the effect of epigenome editing tools in the context of Central Nervous System inflammation

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Multiple Sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) that primarily affects young adults resulting in unpredictable progressive disability. We have shown that epigenetic mechanisms such as DNA methylation, the addition of methyl group on the 5-Carbon of cytosines primarily on CpG dinucleotides without changes in the DNA sequence, plays a role in MS pathogenesis. We aim to functionally investigate the DNA methylation changes identified in blood immune, neuronal and glial cells of MS patients compared to control individuals by exploiting the

potential of CRISPR-dCas9-based epigenome editing to modulate gene methylation and expression. To this aim, we have developed tools that rely on the fusion of dCas9 to the catalytic domain of DNMT3A, a DNA methylating enzyme. In addition to the in vitro validation of the dCas9-DNMT3A systems, we have developed a Cre-dependent dCas9-DNMT3A-EGFP knock-in mouse line. Gene expression analysis of the dCas9-DNMT3A mRNA, using RT-qPCR, indicated a negligible leakage across the tissues in both heterozygous (Dnmt3a+/-) and homozygous (Dnmt3a+/+) knock-in animals. We next induced the expression of the cassette in the brain in vivo by delivering Cre-expressing Adeno-associated viruses (AAVs) in the striatum with contralateral injection of mCherry AAVs serving as control. Analysis of dCas9-DNMT3A expression by RT-qPCR and EGFP reporter protein expression by immunofluorescence revealed an increased expression of the cassette in the ipsilateral striatum following Cre-containing AAV delivery compared to the contralateral one. Moreover, we evaluated the clinical outcome of the different mouse strains using the MS-like model of Experimental Autoimmune Encephalomyelitis (EAE). Our main aim is now to target genes in the neuronal and immune compartments which corresponding orthologs have been found to be differentially methylated in MS patients. We focus on the H2-Ab1 gene, the HLA-DRB1 ortholog in mice, highly expressed in antigen presenting cells, and we have performed ex vivo experiments on bone marrow-derived dendritic cells and macrophages to downregulate the expression of the gene using gRNAs that target the locus. Regarding the CNS, we target the Cnr1 locus which is highly expressed in the striatum. These on-going studies will help us decipher the role of methylation in the inflamed brain and pave the way for new therapeutic strategies.

Keyword: *Multiple sclerosis, Epigenome editing, DNA methylation*

#40 Chronic Traumatic Encephalopathy (CTE) Neuropathology in Athletes and Military Personnel

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OBJECTIVE: To examine the evidence for neuroimmunologic mechanisms that may be involved in the neuropathologic manifestations characteristic of Repetitive Traumatic Brain Injury (TBI) and Chronic Traumatic Encephalopathy (CTE) in Athletes and Military Personnel.

METHODS: We conducted a systematic literature search through the Saint James School of Medicine Library resources as well as PubMed and EBSCO electronic databases. The text words, “concussion”, “high-impact head injury”, “repetitive traumatic brain injury (TBI)”, “chronic traumatic encephalopathy (CTE)”, “tauopathy”, "microglial activation", "neuroinflammation" with the use of the Boolean operator AND “sports”, “football”, “boxer”, “high-impact sports”, “contact sports” “athletes”, “young athlete”, “suicide”, “military personal”, “military veteran”, “blast explosive”, “roadside bomb”, “blast exposure”, “blast neurotrauma” were used to identify relevant studies discussing the clinical and neuropathlogical manifestations in TBI and CTE. Inclusion criteria were the following: 1) must be a scholarly or peer-reviewed source, 2) a relevant article within the last 15 years, and 3) articles published in the English language only. Outcome measures included neuropathologic evidence of neurodegeneration and/or neuroinflammation in the risk groups.

RESULTS: 40 studies were included from the US, Canada, Mexico, Germany, Italy, and the UK. The neuropathologic findings of CTE described in brain autopsies of contact sports athletes and military personnel have been used to develop criteria for Stage I, II, III, and IV based on the severity of neurodegeneration. Severity of CTE progression was shown to be linked to player position and years played. Brain atrophy was documented in the frontal cortex, temporal cortex, medial temporal lobe structures including the hippocampus and amygdala. Microscopic findings included phosphorylated-tau neurofibrillary tangles, beta-amyloid plaques, thread and dot-like neurites, demyelination, axonal loss and Lewy bodies. A higher repetitive head injury index was also correlated with higher axial diffusivity on brain imaging, with major damage occurring in the CC1 area of the corpus callosum. Experimental evidence demonstrated the kinetics of the neuroimmunological response to head trauma including microglial activation, expression of pro-inflammatory markers, and lymphocyte trafficking of CD4+ T cell populations followed by CD8+ T cells.

CONCLUSION: For clinical manifestations of CTE to be present, there must be repeated sublethal trauma. Neuropathologic lesions involve the whole brain with variable spread throughout the parietal, temporal, occipital, and frontal cortex. Recommendations for prevention of CTE include the use of padded helmets. Tests for examining the neuropsychiatric and behavioral symptoms of CTE include the Cumulative Head Impact Index, Trail Making Test, Controlled Oral Word Association Test, Stroop Interference Test, Behavioral Regulation Index, and Behavior Rating Inventory of Executive Function. Potential biomarkers for CTE include Neurofilament Light, Ubiquitin carboxyl-terminal hydrolase L1, GFAP and TNF-a. Provision of supportive treatments for PTSD, depression, aggression, and other neurologic deficits is crucial. Neuroimmunological mechanisms are implicated in triggering neurodegenerative pathology in CTE. Future research is needed to quantify the traumatic load in contact sport athletes and military personnel exposed to TBI, differentiate the risk of long-term neurological consequences for contact sport players who perform individually or in different field positions in team sports, develop monitoring practices for populations at risk, correlate clinical and neuropsychological progression with radiological findings obtained from functional neuroimaging, and research novel stem cell therapy and other treatment strategies for neuroregeneration.

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Keyword: *Repetitive Traumatic Brain Injury (TBI), Chronic Traumatic Encephalopathy (CTE), Athletes, Military Personnel, Neurodegeneration*

#49 NEUROVASCULAR AND IMMUNE DETERMINANTS OF SELECTIVE VULNERABILITY OF DOPAMINERGIC NEURONS IN NON-HUMAN PRIMATES

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Dopaminergic neurons in the ventral tier of the substantia nigra pars compacta (SNc) degenerate prominently in Parkinson's disease (PD), while those in the dorsal tier and ventral tegmental area are relatively spared. The factors determining why these neurons are vulnerable while others not, are still unknown. Neuroinflammation and immune cells infiltration have been demonstrated to be a key feature of PD. Vascular pathology has also been suggested to be another possible contributing factor to disease. However, the link between selective dopaminergic neurons vulnerability, glial and immune cells response, vascular alteration and how these interactions contribute to the overall neuroinflammatory and neurodegenerative process in PD remains unclear. We aimed to investigate the contribution of glial cells activation and immune cells infiltration in the selective vulnerability of ventral dopaminergic neurons within the midbrain in a non-human primate model of PD induced by systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Structural and functional alterations of the vasculature within specific regions of the midbrain have been also evaluated. MPTP-treated monkeys showed a large microglial and astroglial activation in the whole midbrain, but no major sub-regional differences were observed. Remarkably, the ventral substantia nigra was physiologically much more vascularized in comparison with the rest of midbrain regions. This feature probably makes this region more susceptible to immune cells infiltration under pathological conditions as a large infiltration of both T and B lymphocytes was

observed in MPTP-treated monkeys in this region. These data suggest that the higher vascular density within the ventral region of the substantia nigra may represent a crucial factor for the different vulnerability of dopaminergic neurons in the midbrain. The increased infiltration of T and B cells in this region could also play a major role in the selective vulnerability of dopaminergic neurons in PD.

Keyword: *substantia nigra, neuroinflammation, vulnerability, Parkinson's disease, immune cells*

#61 Blood-borne immune cells in the central nervous system: high-dimensional single cell characterization of regional heterogeneity in multiple sclerosis

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Background: Characterization of immune compartments in different regions of the central nervous system (CNS) is essential to understand brain immunity and its dysregulation. However, in multiple sclerosis (MS), although brain microglia regional heterogeneity has been well studied, the CNS regional heterogeneity of blood-borne immune cells remain poorly explored.
Objectives:
1) To characterize the regional heterogeneity of blood-borne immune cells in the CNS of patients with MS at a high- dimensional single cell level.
2) To define the population of blood-borne immune cells within the CNS that are dysregulated in MS pathology.

Methods: The pia, choroid plexus, corpus callosum, cerebral cortex, cerebral white matter and cerebellum from fresh brain, as well as post-mortem peripheral blood was obtained from two MS patients and four patients with other neurological diseases (OND) including amyotrophic lateral sclerosis (n=3) and hereditary spastic paraplegia (n=1). Tissue was processed to obtain a single cell suspension of immune cells, and cells were stained with 21-color flow cytometry panel developed to extensively characterize different immune cells and their phenotype. To extensively study the regional immune cell heterogeneity, unsupervised clustering and data vizualization was performed with FlowSOM, UMAP, and Multiscale PHATE algorithms. Cells and clusters identified in MS patients were then compared with those observed in OND patients to characterize the MS-specific immune signature and heterogeneity across CNS regions.

Results: After excluding doublets, dead cells, and microglia we have successfully retrieved more than 400 000 immune cells from 61 brain regions (230 686 cells from MS, 185 976 cells from OND). In a preliminary analysis of a subset of the dataset including pia and post-mortem peripheral immune cells, we have shown that we can characterize immune cell populations and their subsets in both compartiments (T cells, B cells, NK cells and dendritic cells). Moreover, we have demonstrated that immune cells residing within the pia show a distinct immunophenotype when compared to peripheral immune cells.

Conclusions: Our high-dimensional single cell characterization of the human brain will allow us to define the regional heterogeneity in CNS of patients with MS and other neurological

disorders. Understanding the distinct immune signatures across brain regions in the MS brain will provide important insights into the physiopathology of the disease.

Keyword: *Multiple Sclerosis, Flow Cytometry, Heterogeneity*

#66 The Impact of Multiple Sclerosis and Neuroinflammation in Inducing Nuclear Pore Complex Defects

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Background: Neuronal damage (neurodegeneration) is the major source of disability in multiple sclerosis (MS). Reducing and preventing neurodegeneration represents the next breakthrough in MS treatment, as no treatments impede disease progression. The nuclear pore complex (NPC), located within the nuclear membrane surrounding the nucleus, allows for nucleocytoplasmic transport, vital for transporting cellular machinery between the nucleus and cytoplasm. NPC abnormalities have been shown to be pathogenic in other neurologic diseases where they underlie neuron damage. Here, we examined the relationship between inflammation and the NPC in the context of MS. We hypothesized that MS-associated inflammation would disrupt the NPC and alter its protein constituents.

Methods: Post-mortem MS and control cortical gray matter were immunostained with Lamin B to examine NPC structure. Primary mouse embryonic neurons were treated with interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha) for 24 hours to mimic inflammatory conditions characteristic of MS. Neurons were stained for protein markers of the NPC, including Lamin B and Nup98, to investigate whether inflammation associated with MS affects the NPC structure. A novel, quantitative 3D imaging method utilizing a computer script was used to characterize abnormalities in the NPC.

Results: First, we identified abnormalities in the nuclear membrane and NPC in pyramidal neurons from MS cortex. Next, using quantitative 3D imaging of the NPC, we found that neurons treated with inflammatory cytokines showed a greater than fivefold increase in the percentage of cells with alterations in Lamin B staining, indicative of an abnormal NPC; greater than 20% in IFN-gamma and TNF-alpha treated compared to 4% in control neurons. Additional proteins of the NPC, such as Nup98, confirmed inflammatory cytokine induced abnormalities, with a greater percentage of neurons exhibiting NPC changes in the IFN-gamma and the TNF-alpha conditions compared to controls.

Conclusion: This study is the first to demonstrate NPC damage in neurons in the context of MS and associated inflammatory conditions. Changes in the structure of the NPC alter normal transport between the nucleus and cytoplasm, which is crucial for mRNA translation and cell viability, thus presenting a novel neurodegenerative mechanism in MS.

Keyword: *Neuroinflammation, Multiple Sclerosis, Nuclear Pore Complex*

#67 MS-patient derived somatic coding mutations in the RNA binding protein Heterogeneous Nuclear Ribonucleoprotein A1 (A1) induce neurodegeneration.

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Background: Multiple Sclerosis (MS) disease progression and disability correlate with neurodegeneration; therefore, there is an urgent need to understand the mechanisms underlying neurodegeneration. We previously identified somatic coding mutations in the RNA binding protein heterogeneous nuclear ribonucleoprotein A1 (A1) derived from MS patients, which may contribute to neurodegeneration. The aim of this project is to characterize the neurodegenerative consequences of two mutations in A1, including one within the nuclear localization sequence of A1 ($A1_{P275S}$) and a second in the prion-like domain of A1 ($A1_{F263S}$) to test the hypothesis that neuronal A1 dysfunction drives neurodegeneration in MS.

Methods: Mouse primary embryonic neurons were transduced with adeno-associated viral vectors expressing wildtype A1 ($A1_{WT}$) or mutant ($A1_{P275S}$, $A1_{F263S}$), the human synapsin 1 neuron promoter, and the fluorescent tracker mCherry. A1 nucleocytoplasmic localization patterns, a phenotypic indicator of A1 dysfunction, were assessed via mCherry expression using ImageJ. Neurons were examined for markers of neurodegeneration using immunocytochemistry, including changes in neuronal neurite length and complexity, and FluoroJade-C, which stains degenerating neurons.

Results: Compared to $A1_{WT}$, examination of A1 staining revealed a 50% increase in nucleocytoplasmic mislocalization (**p<0.01) in neurons transduced with $A1_{P275S}$, but not $A1_{F263S}$. While $A1_{F263S}$ did not demonstrate significant mislocalization, both $A1_{F263S}$ and $A1_{P275S}$ induced neurodegeneration, which was evident by significant reductions in total neuronal length by 25% (*p<0.05) and 41% (**p<0.01), respectively; and neuronal complexity by 25% (*p<0.05) and 43%(*p<0.05), respectively, as compared to $A1_{WT}$ transduced neurons. Further, preliminary analysis showed both A1 mutants demonstrated 2.5 fold increase in FluoroJade-C staining.

Conclusions: These results reveal that while MS-patient-derived mutations in A1 may manifest themselves through different phenotypes (i.e., mislocalization vs no mislocalization), both negatively impact neuronal health leading to neurodegeneration. This study establishes a framework for understanding A1 dysfunction as a contributor to neurodegeneration in MS.

Keyword: *Multiple Sclerosis, Neurodegeneration, RNA Binding Proteins*

#80 A novel preclinical model of primary progressive multiple sclerosis based on a familial Nr1h3 mutation presents with a more pathogenic T cell and myeloid phenotype and a failure to recover from disease

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Introduction: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) that often develops to a highly disabling progressive phase lacking treatments. To recapitulate progression, we developed a novel mouse model with a p.Arg413Gln mutation in nuclear receptor subfamily 1 group H member 3 (Nr1h3) homologous to a risk variant in families with rapidly progressive MS. Given the role of NR1H3 and its targets in lipid transport and immune modulation, we aim to define mechanisms driving MS risk, severity, and progression.

Methods: Transcriptome sequencing, immunohistochemistry, and flow cytometry were used to assess peripheral immune cells in heterozygous (HET) and homozygous (HOM) Nr1h3 mutant vs wild-type (WT) mice. Mice were induced with myelin oligodendrocyte glycoprotein (MOG_{35-55}) experimental autoimmune encephalomyelitis (EAE) preclinical model of MS and assessed for disability and histology at peak (d17-18) and chronic (d50) EAE. Inflammatory cytokines were evaluated in ex vivo MOG-challenged lymph node and splenic T cell supernatants at pre-onset (d10) and peak EAE, and flow cytometry was used to evaluate CNS resident and infiltrating cells at peak EAE.

Results: Naive Nr1h3 HET and HOM vs WT mice had lower splenic expression of CD163 and CD209b myeloid reparative proteins and CD5 antigen-like, a modulator of lipid homeostasis, myeloid repair, and T cell pathogenicity. In EAE, mutant vs WT mice at peak had no difference in disease severity or spinal cord histology. However, HET mice had increased demyelination ($p=0.003$), axonal damage ($p=0.02$), leukocyte infiltration ($p=0.03$), and macrophage/microglia activation ($p=0.01$) in the cerebellar white matter or medulla. In the brain, HET mice had reduced numbers of CD4+ T cells producing IL-10 ($p=0.007$) and IL-22 ($p=0.02$) CNS-protective cytokines and myeloid cells producing CD163 ($p<0.05$). HET mice had increased MOG-reactive lymph node T cell production of IL17, TNFa, and IFNy ($p<=0.05$) pre-EAE onset and increased splenic T cell production of IL17, TNFa, and IL22 ($p<=0.03$) at peak EAE. At chronic EAE, HOM vs WT mice had greater disability without recovery and greater spinal cord pathology with more lipid deposits, pointing to macrophage failure to repair lesions.

Conclusion: The Nr1h3 model shows a more pathogenic T cell and myeloid phenotype, increased disability, neurodegeneration, and reduced ability to repair, elucidating key mechanisms underlying aggressive and progressive MS.

Keyword: *Multiple sclerosis, Experimental models, Neuroimmunology, Neurodegeneration*

#86 Role of microglia and aging during oxidized phosphatidylcholine induced chronic neurodegeneration

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Chronic neurodegeneration and aging are critical processes associated with multiple sclerosis (MS) progression. Effective treatment for progressive MS is lacking because we do not fully understand the mechanisms that drive chronic neurodegeneration and why it is accelerated by aging. Analysis of post-mortem MS tissues found lesions with chronic neurodegeneration have elevated reactive microglia and oxidative stress signatures including oxidized phosphatidylcholines (OxPC). Thus, we stereotaxically injected OxPC into the mouse spinal cord (SC) to model MS relevant oxidative injury and found OxPC are potent mediators of

acute neurodegeneration that required neutralization by microglia (1). Moreover, aging dysregulates microglia function and exacerbates OxPC mediated neurodegeneration (2). Since these studies mainly focused on the first 7 days of the OxPC lesion, we next aimed to investigate the relationship between OxPC, microglia, and aging during chronic neurodegeneration. To assess OxPC mediated chronic neurodegeneration, we analyzed OxPC lesions from young and middle-aged mice 42 days after the initial injection and found unremitting inflammation, demyelination, and axon loss. More importantly, endogenous OxPC deposition and chronic neurodegeneration were exacerbated in middle-aged mice compared to young mice. Using tamoxifen pretreated $CX3CR1^{CreERT}$:$Ai9^{TdTomato}$ mice, we found microglia were only approximately 50% of all $IBA1^{+}$ macrophages in chronic OxPC lesions of young mice, suggesting they may be replaced by peripheral monocyte derived macrophages. Notably, chronic OxPC lesions from middle-aged mice had less $TdTomato^{+}$ cells compared to lesions from young mice, suggesting that aging reduced the microglia response to chronic OxPC lesions. Young tamoxifen pretreated $CX3CR1^{CreERT}$:$ROSA26^{iDTR}$ mice were also injected with PBS or with diphtheria toxin from days 35-42 to determine if microglia were beneficial or harmful in chronic OxPC lesions. Mimicking the aging chronic lesions, microglia depletion in these mice also caused greater axonal injury and endogenous OxPC deposition. Collectively, these results highlight the ability of OxPC to sustain chronic neurodegeneration, which was exacerbated by aging and microglia loss. Further investigations into the molecular mechanisms of this model will help to understand how aging dysregulates chronic oxidative injury, neurodegeneration, and MS progression.

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Keyword: *Aging, Microglia, Multiple scleorsis, Neurodegeneration, Oxidized phosphatidylcholine*

#99 Age-related impaired remyelination is associated with dysregulated microglial transitions

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Introduction: Multiple sclerosis (MS) is a neurodegenerative condition that is characterized by multiple, focal demyelinating lesions. Within these lesions, lost myelin may be regenerated through remyelination, where greater remyelination in people with MS correlates with reduced disability. However, remyelination is highly variable, prone to failure, and becomes less efficient with age. Remyelination is promoted by microglia via the

secretion of pro-remyelination factors and clearance of inhibitory molecules. Microglia form distinct states specific to demyelination and the early stages of remyelination, but it remains unclear how microglia change throughout remyelination, specifically in young animals where remyelination is efficient and middle-aged animals where remyelination is delayed. **We hypothesized that distinct microglial states are specific to remyelination and are altered with age.**

Methods: We used the lysolecithin (LPC) mouse model wherein LPC is injected into the spinal cord to induce demyelination followed by robust remyelination. We isolated microglia from LPC-lesioned spinal cords and used single-cell RNA sequencing coupled with advanced bioinformatics techniques to characterize microglial subpopulations.

Results: We identified distinct remyelination-associated microglia (ReAM) states present throughout remyelination that we named according to differentially expressed gene. In young mice Igf1-ReAM, Ccl3-ReAM, and Irf7-ReAM were present at 7 days after LPC injection (DPI) corresponding to early oligodendrocyte production. Plp1-ReAM were present during late stages of remyelination, at a time point where microglia engulfed newly produced oligodendrocytes and myelin. Igf1-ReAM displayed enhanced metabolic activity. In middle-aged mice we found a dysregulated microglial response with a shift in many of these ReAM states. While Irf7-ReAM were found at 7 DPI, like with young mice, Igf1-ReAM were delayed and Plp1-ReAM were virtually absent from in middle-aged mice, consistent with impaired remyelination.

Conclusion: Taken together, we identified distinct microglial states throughout remyelination. Age-related remyelination decline is associated with a dysregulated temporal sequence of ReAM states. Promoting the transitions between these microglial states may promote remyelination in people with MS.

Keyword: *Microglia, Multiple Sclerosis, Heterogeneity, Aging, SIngle-cell RNA sequencing*

#113 Effects of Alzheimer's Disease on Inflammasome Activation Following Traumatic Brain Injury

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Traumatic brain injury (TBI) is a risk factor for the development of Alzheimer's disease (AD) and Alzheimer's Disease Related Dementias (AD/ADRD). We have previously shown that the inflammasome is a key contributor to the pathophysiology of traumatic brain injury (TBI). Moreover, the inflammasome also plays a significant role in the pathology of AD. The inflammasome is a multiprotein complex involved in the activation of caspase-1, the processing of the pro-inflammatory cytokines IL-1beta and IL-18, and the programmed cell death process of pyroptosis. In this study, we aimed to determine the effects of TBI on inflammasome activation in the brain of 5-month-old mice with a genetic predisposition towards AD (3xTg) and wild-type (WT) controls by using the TBI model of controlled cortical impact (CCI) to study the effects of inflammasome activation in the brain acutely and chronically after TBI in WT and AD mice with and without TBI. Inflammasome signaling activation was assessed, and our findings indicate that acutely after TBI, there was increased expression of the inflammasome proteins apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC),

caspases-1 and -8, and IL-1beta in WT and 3xTg (AD) animals. Moreover, when comparing AD-TBI mice vs. WT-TBI mice, we found increase expression of ASC and IL-1beta in the brain of AD-TBI mice, consistent with altered cognitive performance and increase amyloid-beta in the AD-TBI group at 12 weeks post-TBI. Furthermore, when comparing the WT-TBI vs AD-TBI mice at 3-months after TBI, we found increased expression of NLRP3, caspase-8 and ASC in the cortex of the WT-AD mice, consistent with increased expression of glial fibrillary acidic protein (GFAP) and neurofilament light (NfL) in the brain of these mice. Importantly, treatment of mice after TBI with a brain-penetrant therapeutic monoclonal antibody against ASC (IC100) decreased inflammasome activation. Together, these findings suggest an important role for the inflammasome after TBI in individuals with a genetic predisposition towards AD and highlight that the inflammasome is a promising therapeutic target for AD/ADRD.

Keyword: *inflammasome, traumatic brain injury, alzheimer's disease, caspase-1, cytokines*

#117 UBE3A promotes foam cell formation and counters remyelination by targeting ABCA1 for proteasomal degradation

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The accumulation of foamy macrophages is a pathological hallmark of metabolic and brain diseases such as multiple sclerosis (MS). Emerging evidence indicates that perturbed metabolism and efflux of intracellular lipids underlies the development of a harmful foamy macrophage phenotype in these disorders. To date, the molecular mechanisms that underlie dysregulation of cellular lipid metabolism are not fully understood. Here, we show that the ubiquitin-proteasome system controls turnover of cholesterol efflux transporter ATP-binding cassette A1 (ABCA1) in lipid-loaded macrophages in the brain. We report that sustained intracellular accumulation of myelin-derived lipids promotes the abundance and activity of ubiquitin-protein E3 ligase A (UBE3A) in macrophages, which in turn stimulates ABCA1 ubiquitination and subsequent degradation. Consequently, UBE3A-mediated ABCA1 degradation boosted cellular lipid accumulation, and induced the formation of an inflammatory macrophage phenotype that impaired remyelination. By using RNA sequencing analysis, we further established Tat-interacting protein 30 (TIP30), an inhibitor of importin β-mediated nuclear import, as an essential regulator of cytosolic UBE3A levels. Collectively, our findings identify UBE3A as a driver of foam cell formation, and indicate that targeting UBE3A-mediated ABCA1 degradation is a promising strategy to mend faulty lipid metabolism in foamy macrophages and enhance central nervous system repair.

Keyword: *Foamy macrophage, ABCA1, Ubiquitin proteasome system, UBE3A, Remyelination*

#118 The multifaceted potential of targeting Elovl6 in phagocytes and oligodendrocytes for central nervous system remyelination

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Phagocytes like macrophages and microglia play a dual role in lesion progression in multiple sclerosis (MS) by promoting both inflammation and remyelination in the central nervous system (CNS). Excessive uptake of myelin by macrophages and microglia during demyelination results in enhanced inflammatory activation of these phagocytes and impairs oligodendrocyte-mediated remyelination. This functional alteration is accompanied by the rewiring of fatty acid elongation by Elovl6 in both phagocytes and oligodendrocytes, marking this enzyme as a possible therapeutic target for decelerating MS lesion progression. Hence, by using in vitro, ex vivo, and in vivo models we studied if interfering with Elovl6 altered inflammation, myelination, and oligodendrocyte precursor cell (OPC) differentiation.

We found that inhibiting myelin-induced upregulation of Elovl6 halts foam cell formation by facilitating ABCA1-mediated cholesterol efflux in phagocytes, reducing inflammation, and enhancing the production of neurotrophic factors in vitro. Similarly, knockout of Elovl6 in OPCs increases the differentiation towards myelin-producing oligodendrocytes and their migration capacity in vitro. Lipidomic analyses revealed metabolic alterations in lipid species underlying these altered phenotypes. These findings were confirmed in ex vivo and in vivo models for remyelination by generating cell-specific and full Elovl6 knockout mice. Phagocyte-specific Elovl6 knockout effectively reduces lipid load and improves CNS repair. This effect is even larger in full Elovl6 knockout, thereby providing evidence that Elovl6 is a multifaceted target that enhances remyelination through the modulation of multiple cell types.
Altogether, our results indicate that metabolic rewiring of FAS and upregulation of Elovl6 underly the inflammatory phenotype of phagocytes and modulates the remyelinating capacity of oligodendrocytes. Hereby, Elovl6 provides a novel therapeutic target for inducing CNS repair in MS.

Keyword: *Lipid metabolism, Remyelination, Multiple sclerosis, Neuroinflammation*

#120 A non-canonical role of Mir155hg in autoimmune demyelination unveiled using a novel lipid nanoparticle-based RNA transfection method

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Mir155hg encodes a microRNA called miR155 and contributes to experimental autoimmune encephalomyelitis (EAE) via an unclear mechanism. Here we report that Mir155hg: 1) is predominantly expressed in lymph node type-2 conventional dendritic cells (cDC2); 2) is crucial in the initial phase of EAE, but dispensable once clinical symptoms appear; and 3) reduces D-

amino acid oxidase mRNA (Dao) independently of miR155. This mechanism was observed in cells transduced with lentiviral vectors expressing DAO and wild-type or mutant Mir155hg, as well as transfected with microfluidic-produced lipid nanoparticles encapsulating miR155 mimic or inhibitor. In sum, this project: 1) clarify the mechanism of action of Mir155hg in EAE; and 2) provides a simple, effective, and non-toxic approach for delivering RNA into monocytic cells in a reproducible manner.

#129 Control of Lymphocyte Inflammation through the Canonical Wnt Pathway Signaling

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Background: Multiple Sclerosis (MS) is an auto immune disease that causes demyelination and destruction of neurons in the central nervous system (CNS) via infiltrating immune cells. During MS, and its animal model, experimental autoimmune encephalomyelitis (EAE), myeloid and lymphoid cells are found in abundance in lesions. Wnt is a family of lipoglycoprotein that bind to Frizzled receptors, leading to inhibition of beta-catenin destruction complexes. Increased beta-catenin allow for activation of Wnt targets involved in neurogenesis in the CNS. Analysis of transcripts associated with MS-like lesions in EAE revealed a significant increase of genes associated with the Wnt beta-catenin pathway. Based on this data, we hypothesize that the Wnt pathway plays an important role in multiple sclerosis via regulation of lymphoid cells.

Results: While we did not see many phenotypic changes on our CD8 T cells upon Wnt stimulation, CD4 T cells showed a significant decrease in some pro-inflammatory cytokines in the resting condition. The CD4 T cell in the activated condition showed an increase in TGF-b, Fas, and ICOS, indicating that these cells are developing more of a regulatory phenotype. With CD19 B cells, we see an increase in regulatory cytokines and a decrease in ALCAM and CD86, leading us to think that these cells seem to be developing more of a regulatory phenotype. In conclusion, we see that the Wnt beta-catenin pathway has the ability to play a regulatory role on lymphocytes, making it a good potential therapeutic in the future for MS.

Keyword: *Lymphocyte, Wnt, T cell, B cell*

#130 Determining the Pathological Role of Peripheral Myeloid Cells in the CNS of mouse model for Alzheimer's Disease

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Background/As therapies targeting amyloid beta plaques have failed to improve symptoms of Alzheimer's disease (AD), the role of neuroinflammation in AD pathology has become increasingly studied. Levels of neuroinflammation have been shown to correlate with disease progression and cognitive impairment, suggesting that neuroinflammation contributes to AD pathogenesis. In the event of abnormalities such as injury or toxic metabolites in the brain, resident cells in the central nervous system (CNS) such as microglia and astrocytes activate and take on an inflammatory phenotype to heal tissue and recruit immune cells from the periphery. While research has shown that resident CNS cells adopt an activated profile during AD, little is known about the role of infiltrating peripheral immune cells. Infiltrating myeloid cells are of particular interest due to results from recent genetic studies.

Objective/Here we establish a time course of infiltrating immune cells during AD progression and characterize their profile in a mouse model of AD.

Methods/Male BLK6SJL/5XFAD mice were used to characterize immune cell infiltration at 6- and 12-month time points.

Results/Our data shows that infiltrating monocytes, macrophages and dendritic cells (DCs) increased their expression of proinflammatory factors IL-1b and TNF-a as AD progresses. We also show that DCs bear a substantial portion of IL-b production.

Conclusions/So far, these findings support our hypothesis that peripheral immune cells play a key role in neuroinflammation and AD pathology.

#131 The ApoA-I mimetic peptide 5A enhances remyelination by promoting clearance and degradation of myelin debris

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The progressive nature of demyelinating diseases lies in the inability of the central nervous system (CNS) to induce proper remyelination. Recently, we and others demonstrated that a dysregulated innate immune response partially underlies failure of CNS remyelination. Extensive accumulation of myelin-derived lipids and an inability to process these lipids was found to induce a disease-promoting phagocyte phenotype. Hence, restoring the ability of these phagocytes to metabolize and efflux myelin-derived lipids represents a promising strategy to promote remyelination. Here, we show that ApoA-I mimetic peptide 5A, a molecule well-known to promote activity of the lipid efflux-transporter ABCA1, markedly enhances remyelination. Mechanistically, we find that repair-inducing properties of 5A are attributed to increased clearance and metabolism of remyelination-inhibiting myelin debris via the fatty acid translocase protein CD36, which was transcriptionally controlled by the ABCA1-JAK2-STAT3 signaling pathway. Altogether, our findings indicate that 5A promotes remyelination by stimulating clearance and degradation of myelin debris.

Keyword: *ApoA-I mimetic peptide 5A, Remyelination, Phagocyte, Lipid droplet degradation, Myelin debris clearance*

#141 Investigating microglial heterogeneity in a LRRK2 genetic mouse model of Parkinson's disease pathology

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Microglia, the central nervous system resident immune cells, are now recognized to critically impact homeostasis maintenance and contribute to the outcomes of various pathological conditions including Parkinson's disease (PD). Microglia are diverse, with different states recently identified in neurodegenerative disease models, including the 'disease-associated microglia' which present a selective enrichment of CLEC7A encoding Dectin-1 protein and the 'dark microglia' (DM) displaying markers of cellular stress at the ultrastructural level. However, the roles of Dectin-1 positive microglia and DM in PD pathology have remained elusive. By applying immunofluorescence and scanning electron microscopy, we aimed to characterize 1) the Dectin-1 positive cell population, and 2) their possible relationships to DM in a mouse model harboring a G2019S pathogenic mutation of the LRRK2 gene, the most common mutation linked to PD. We compared 18-month-old female wild type and G2019S mutation LRRK2 knockin mice. In the dorsal stratum, a region affected by PD pathology, immunofluorescence analysis revealed higher number of Dectin-1 positive cells and a selective enrichment of ameboid morphology in Dectin-1 positive cells from Lrrk2 G2019S mice versus controls. Furthermore, extensive ultrastructural features of stress (e.g., endoplasmic reticulum and Golgi apparatus dilation), as well as reduced intact cellular contacts, were observed in microglia from LRRK2 G2019S mice versus controls. Dectin-1 positive microglia exhibited extensive phagocytic ultrastructural characteristics in the LRRK2 G2019S mice. Lastly, the LRRK2 G2019S mice presented a higher proportion of DM. In summary, we provide novel insights into recently-defined microglial states, Dectin-1 positive cells and DM, in the context of LRRK2 G2019S PD pathology.

Keyword: *Neurodegenerative diseases, Microglia, Dark Microglia, Parkinson's disease, Scanning electron Microscopy*

#143 Characterization of BTK and pBTK expression in lesions of MS and its mouse models

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The use of anti-CD20 B-cell depletion therapy has shown high efficacy in the treatment of relapsing-remitting multiple sclerosis (MS). Thus, there is interest in additional ways to inhibit B-cell activity. Medications in MS should also aim to reduce the inflammatory and neurotoxic properties of infiltrating monocytes/macrophages and tissue-resident microglia. Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase that is found within immune cells, including B-cells and myeloid cells such as macrophages and microglia. It is critical in the maturation and activation of B cells through the B-cell receptor and involved in the activation of myeloid cells through toll-like receptors and Fc

receptors. Thus, BTK inhibition has the potential as a dual-pronged approach to reduce inflammatory activities of both B-cells and myeloid cells in MS. Despite interest in the field, and eleven ongoing Phase III clinical trials in MS, the spatial localization and extent of BTK and activated BTK (pBTK) expression in lesions of MS and its models are not well characterized. Thus, we hypothesized that BTK and pBTK would be persistently elevated in microglia/macrophages in CNS lesions of MS brain and mouse models. Using fluorescent immunohistochemistry, we observed widespread expression of BTK and pBTK in MS brain samples containing chronic active lesions, corresponding predominantly to Iba1-expressing microglia/macrophages shown by Imaris 3D-rendering of co-localization. In mouse spinal cord lesions inflicted by the local injection of oxidized phosphatidylcholine (OxPC) or lysolecithin, BTK and pBTK are localized to lesions correspondent with CD68-positive microglia/macrophages, and not in contralateral normal-appearing white matter (NAWM). BTK and pBTK expression are steadily elevated from day 3 of the injury onward to 21 days post-demyelination. In the experimental autoimmune encephalomyelitis (EAE) model in mice during peak clinical severity and chronic phase of the disease, BTK and pBTK signals are widespread in the spinal cord but are restricted to lesions compared to NAWM. Overall, our results show that microglia and macrophages recruited to the injury upregulate and maintain their expression of BTK and pBTK in CNS lesions of MS and its mouse models.

Keyword: *Bruton's Tyrosine Kinase, MS Pathology, Multiple Sclerosis, Animal Model Pathology*

#150 The β2-Adrenergic Receptor Agonist Terbutaline Upregulates T Helper-17 Cells in a Protein Kinase A-Dependent Manner.

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Background: T helper 17 (Th17) cells produce IL-17A cytokine and can exacerbate autoimmune neuroinflammatory conditions such as multiple sclerosis[1]. The β2 adrenergic receptor is a g protein-coupled receptor that induces cAMP second messenger pathways[2]. We tested the hypothesis that terbutaline, a β2-adrenergic receptor-specific agonist, promotes IL-17 secretion by memory Th17 cells in a cAMP and PKA-dependent manner.

Methods: Venous peripheral blood mononuclear cells from healthy human participants were activated ex vivo with anti-CD3 and anti-CD28 antibodies[3]. Memory CD4+CD45RO+CD45RA- T cells were obtained from mononuclear cells by immunomagnetic negative selection. The effects of terbutaline, PKA inhibitors H89 and Rp-cAMP, and phosphodiesterase inhibitor rolipram were

tested. IL-17A was measured in the cell culture supernatants with enzyme linked immunosorbent assay. Intracellular IL-17A, IFNgamma, and RORgamma were measured using flow cytometry, CREB, and phosphorylated-serine133-CREB were measured using by western blotting memory Th cells.

Results: Terbutaline increased IL-17A ($P < 0.001$) and increased the proportion of Th17 cells ($p < 0.05$) in activated peripheral blood mononuclear cells and memory Th cells. The PKA inhibitors H89 ($p < 0.001$) and Rp-cAMP ($p < 0.01$) abrogated the effects of terbutaline on IL-17A secretion. Rolipram increased IL-17A ($p < 0.01$) to a similar extent as terbutaline. Phosphorylated-Serine133-CREB was increased by terbutaline ($p < 0.05$), indicating that this transcription factor was activated within Th17 cells. Conclusion: We demonstrated that terbutaline augments memory Th17 cells in lymphocytes from healthy participants. This data suggests that adrenergic stimulators could exacerbate autoimmune diseases in cases where Th17 cells are considered to be pro-inflammatory.

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Keyword: *Autoimmune, multiple sclerosis, Th17 cells, adrenergic receptor, terbutaline*

#152 Inhibiting nighttime melatonin and boosting cortisol increase patrolling monocytes, phagocytosis, and myelination in a murine model of multiple sclerosis

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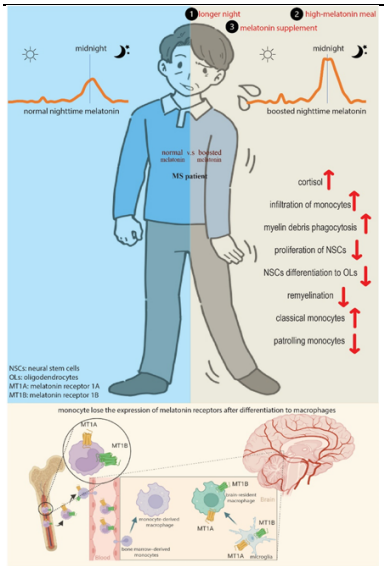

Background: Conflicting results are reported concerning melatonin synthesis in multiple sclerosis (MS) likely due to variabilities between patients' lifestyles, not considered when supplementing melatonin. Here, we aimed to

investigate the melatonin/cortisol alterations by manipulation of circadian light:night cycle and exogenous melatonin.

Methods: The cuprizone model was used which mimics several characteristics of progressive MS. Synthesis of both circadian melatonin and cortisol were manipulated by our novel approach of light-darkness. In addition, since melatonin acts through its receptors, we used the receptor agonist, melatonin, and antagonist, luzindole, while we identified the receptor expression in our model. Furthermore, chimeric mice were used to discriminate bone marrow (BM)-derived macrophages from brain-resident macrophages and also for examination of infiltration. We further studied neural stem/progenitor cells (NSPCs) proliferation, differentiation to oligodendrocyte precursor cells (OPCs), and their recruitment to the site of demyelination and maturation to oligodendrocytes (OLs). Furthermore, monocyte subtypes and macrophages were also evaluated.

Results: We identified melatonin receptors in: OLs of the corpus callosum, where demyelination happens; subventricular zone, where NSPCs are located, and choroid plexus, where it functions as a blood-cerebrospinal fluid barrier. Moreover, residential macrophages were found to express melatonin receptors, whereas BM derived macrophages lose this expression in the demyelinated brain. Next, we showed that cuprizone-fed mice tend to increase melatonin levels. While we employed different approaches to alter circadian rhythm of melatonin and cortisol, only the constant light approach increased cortisol level, NSPC's proliferation and differentiation to OPCs, their maturation to OLs and recruitment to the site of demyelination, the number of patrolling monocytes, and phagocytosis. In contrast, constant darkness and exogenous melatonin exacerbated all these events and amplified infiltration of monocytes.

Conclusions: Melatonin should not be considered as a universal remedy, as currently claimed. Boosting melatonin levels, to a value higher than its normal level, in autoimmune diseases including MS amplifies the immune system function and exacerbate the diseases. Our data emphasize the importance of monitoring melatonin/cortisol oscillation in each MS patient, to avoid melatonin's overdose.

Keyword: *multiple sclerosis, melatonin, monocyte, day/night, demyelination*

#157 Elevated expression of α5-integrin by myeloid cells in motor areas provides a potential target for therapeutics in ALS.

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Amyotrophic lateral sclerosis (ALS) is a fatal disease affecting upper and lower motor neurons and leading to progressive paralysis. While motor neurons are the main cells affected in ALS, the microglial cells, the macrophages of the central nervous system, and peripheral macrophages in the nerve react strongly to the disease and become reactive. Previous studies have shown that microglial cells influence the progression of

the disease by maintaining inflammation and interacting directly and indirectly with the motor neurons. In addition, modulating microglial cells and peripheral nerve macrophage profiles have been shown to influence disease progression. In a previous study from our lab, single-cell mass cytometry (CyTOF) analysis revealed a prominent expression of α5 integrin in microglia and macrophages in a superoxide dismutase-1 G93A mouse model of ALS ($SOD1^{G93A}$). Our new analysis revealed that α5 integrin- positive microglial cells and sciatic nerve macrophages display a very inflammatory phenotype. Interestingly, in post-mortem tissues from ALS patients with various clinical ALS phenotypes and disease duration, α5 integrin was expressed in motor pathways of the central and peripheral nervous system and highly upregulated compared to controls, making it a relevant target to modulate microglial cell and macrophage inflammatory profiles in ALS. In an attempt to assess the downregulation of alpha 5 as a potential therapeutic target for ALS, we administered a monoclonal antibody against α5 integrin to $SOD1^{G93A}$ mice. Targeting α5 integrin in $SOD1^{G93A}$ mice, reduced microglial cell reactivity, improved motor functions and increased survival compared to an isotype control. Together these findings in mice and humans suggest that α5 integrin is a potential therapeutic target for ALS.

Keyword: *Amyotrophic Lateral Sclerosis, microglial cell, Neuroinflammation, Integrin, Therapeutic*

#158 Altered expression of chemokine CXCL12 and its atypical chemokine receptor 3 in multiple sclerosis lesions

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Chemokines and their receptor interactions play crucial roles in the recruitment and activation of immune cells during the pathogenesis of the chronic neuroinflammatory disease multiple sclerosis (MS). Atypical chemokine receptor 3 (ACKR3; previously known as CXCR7) acts as an atypical chemokine receptor by internalizing and sequestering its ligand CXCL12, thereby regulating its availability and downstream signaling via CXCR4. CXCR4 is expressed on immune cells, including monocytes, CD4+ and CD8+ T cells, and B cells. Together these interactions contribute to the migration of these cells across the blood-brain barrier (BBB) and into the central nervous system (CNS) in MS. Animal studies have shown an essential role for ACKR3 in controlling abluminal CXCL12 as well as a protective role for parenchymal CXCL12 in promoting remyelination. levels, However, the regulation of CXCL12 and ACKR3 in human MS lesions is yet not defined. To elucidate the role of this pathway in MS, we aim to delineate the cell-specific production sites of CXCL12 as well as it protein distribution, and the expression pattern of ACKR3 in well-defined post-mortem MS brain tissue and non-neurological controls. Using immunohistochemistry, we found a significant increase of CXCL12 protein in the parenchyma and around CD45+ cells in both active as well as inactive MS lesions and a gradient from the endothelium into the parenchyma. Using in situ hybridization, we detected increased CXCL12 RNA production by endothelial cells in MS lesions compared to control tissue and normal appearing white matter. Additionally, ACKR3 protein levels

were decreased in endothelial cells and astrocytes, suggesting that the increased CXCL12 protein in MS lesions is caused by both higher RNA production as well as decreased scavenging by ACKR3. Restoring the abluminal-to-luminal CXCL12 balance with the use of small molecule ACKR3 compounds may hold promise for mitigating neuroinflammation, inhibiting demyelination and promoting neuronal survival.

Keyword: *neuroimmunology, chemokine, atypical chemokine receptor, blood-brain barrier, multiple sclerosis*

#165 Gasdermin B activation in glia drives to cell death in progressive multiple sclerosis

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Background: Progressive multiple sclerosis (P-MS) is an inflammatory disease of the central nervous system (CNS), defined by physical/mental disabilities, underpinned by inflammatory demyelination accompanied by axonal injury and neuronal death. Previous studies have implicated inflammasome activation and inflammatory regulated cell death, termed pyroptosis ("fiery death") in myeloid cells including macrophages and microglia. Gasdermin B (GSDMB) is a unique member of the gasdermin family that is selectively expressed in humans and undergoes proteolytic cleavage by caspase-1 or granzyme A to cause pyroptosis. Herein, we investigated GSDMB expression in neural cells and its contribution(s) to cell death in P-MS.

Methods: Human autopsied CNS tissues, human primary and immortalized glial cell cultures were investigated by RNASeq, RT-PCR, western blotting, cell death assays and immunolabeling.

Results: We observed that GSDMB and CASP1 were induced in P-MS cerebral white matter compared to nonMS brain tissues, based on RNASeq and RT-PCR analyses while immunodetection revealed GSDMB to be expressed in oligodendrocytes and astrocytes in P-MS brains. Western blotting disclosed the presence of proteolytically cleaved GSDMB in white matter from P-MS patients. Cleavage of GSDMB by caspase-1 resulted in a N-terminus moiety (N-GSDMB) that was apparent in interferon-g exposed astrocytes. Expression of N-GSDMB by transfecting astrocytes and oligodendrocytes (MO3.13) caused pyroptosis, evidenced by increased LDH release, in contrast to the transfected full-length or C-terminus of GSDMB. GSDMB overexpression in oligodendrocytes resulted in decreased myelin basic protein (MBP) expression.

Conclusions: GSDMB was principally expressed in astrocytes and oligodendrocytes within human brain while its N-terminus caused pyroptosis in glial cells. Glial death caused by GSDMB might represent a critical mediator of neuroinflammation and demyelination in P-MS.

#166 In vivo tracking of the cellular contributors to remyelination in the inflamed mammalian cortex

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Remyelination, the endogenous repair of myelin sheaths in the central nervous system (CNS), is a promising therapeutic target for demyelinating diseases like multiple sclerosis (MS). However, remyelination occurs only inconsistently in patients with MS and often remains incomplete. In order to harness this endogenous repair potential therapeutically, it is crucial to unravel its mechanistic underpinnings and define the cell types responsible for it. It was assumed that remyelination solely relied on the formation of new myelin sheaths by newly differentiating oligodendrocyte precursor cells (OPC). However, recent studies have challenged this idea by proposing that mature oligodendrocytes (OL), which manage to survive the inflammatory attack, can provide a substantial contribution to the repair of demyelinated lesions.

We tackled this question by investigating the fate of mature and newly differentiating oligodendrocytes and their corresponding myelin sheaths in a mouse model of cortical MS (c-MS). This model of inflammatory, cortical demyelination resembles sub-pial gray matter lesions in MS. We used a conditional transgenic strategy to selectively label mature OLs. With in-vivo microscopy, we could then observe sparsely labeled mature OLs over the course of cortical de- and remyelination in the subpial gray matter. We showed that in the recovery phase of c-MS, mature, surviving OLs would indeed show the capacity to form new primary processes and to create new internodes. This process, however, was often inefficient and even in the recovery stage, concurrent demyelination outweighed the ongoing remyelination by mature OLs. In the same time OPCs proliferated and efficiently created new myelin sheaths over the course of c-MS. Notably, treatment with the remyelination-promoting drugs clemastine and metformin boosted the overall myelin recovery without affecting the contribution from mature OLs.

Taken together, our results show that, while damaged oligodendrocytes can survive long term in the inflamed CNS and show abortive signs of regeneration, they ultimately fail to make a consequential contribution to myelin recovery.

To further examine the molecular regulation of myelin repair in neuroinflammatory lesions, we are currently performing a single nuclear transcriptomic analysis of newly differentiating and surviving oligodendrocytes over the course of gray matter demyelination and remyelination.

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Keyword: *in vivo microscopy, multiple sclerosis, oligodendrocyte precursor cell, oligodendrocytes, remyelination*

#167 Gasdermin D activation in oligodendrocytes and microglia drives inflammatory demyelination in progressive multiple sclerosis

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Neuroinflammation, demyelination, and neuro-axonal injury are hallmarks of progressive multiple sclerosis (P-MS). A better understanding of the underlying neuroinflammatory processes that occur is vital to develop both novel therapeutics and better diagnostic tools for P-MS. The gasdermin family are proteins involved in programmed inflammatory cell death, particularly pyroptosis, the best studied of which is gasdermin D (GSDMD). Herein, we investigated the actions of GSDMD in P-MS using three experimental platforms. Frontal white matter from persons with P-MS showed induction of inflammasome and pyroptosis genes within demyelinating lesions compared to nonMS (control) white matter. Furthermore, in tissues from persons with MS, there was colocalization of GSDMD expression with Iba-1, signifying its presence in activated microglia, and with GST-pi, a marker for oligodendrocytes. Conditioned medium from GSDMD$^{+/+}$ human myeloid cells caused cytotoxicity of cultured human oligodendrocyte and neuronal cells compared to medium from GSDMD$^{-/-}$ cells. Iba-1 immunopositive microglia displayed marked GSDMD immunoreactivity in the central corpus callosum of cuprizone (CPZ)-exposed Gsdmd$^{+/+}$ mice that was associated with diminished myelinated axonal and OPC counts. Electron microscopy showed an increase in myelin G-ratios in Gsdmd$^{+/+}$ compared to Gsdmd$^{-/-}$ animals. There were also more myelinated axons and total axon counts in Gsdmd$^{-/-}$ mice after CPZ exposure compared to Gsdmd$^{+/+}$ mice. ^{18}FDG PET imaging displayed increased glucose metabolism in the hippocampus and whole brain with preserved neurobehavioral performance in Gsdmd$^{-/-}$ animals after CPZ exposure. These data indicated that GSDMD activation in CNS glia promoted demyelination and neuroaxonal injury in P-MS, offering new insights for treating P-MS.

Keyword: *Multiple Sclerosis, Gasdermin D, Neuroinflammation, Oligodendrocytes, Cuprizone*

#168 Expression and role of Toll-like receptors in facial nerve regeneration after facial nerve injury

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Objective: There are currently no known treatment methods for ensuring complete recovery from facial nerve damage. Thus, various basic and clinical studies continue to be conducted to achieve this end. As part of this research, Toll-like receptors (TLRs), which are associated with innate immunity, are being investigated for their role in nerve regeneration. Here, we reviewed the existing literature regarding the involvement and significance of TLRs in facial nerve injury and regeneration.

Methods: A comprehensive literature review was conducted to assess the expression and role of TLRs in facial nerve injury and subsequent regeneration.

Results: Studies conducted on rats and mice have demonstrated expression of TLR1–13. Among these, TLR2, -3, -4, -5, and -7 were most extensively studied in relation to facial nerve degeneration and regeneration.

Conclusion: This analysis indicates that TLRs are involved in the process of nerve regeneration following facial nerve damage. Inadequate TLR expression or absence of TLR responses can result in regeneration issues after facial nerve damage. Animal studies suggest that TLRs play an important role in facial nerve degeneration and regeneration.

Keyword: *Facial nerve, Toll-like receptor*

#177 Dysregulated intestinal neuro-immune axis underlying early Parkinson's disease symptoms

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Decades prior to clinical diagnosis, patients with Parkinson's disease (PD) often manifest non-motor symptoms, including constipation. Notably, intestinal inflammation and infection positively correlate with incidence of PD. Little is known nonetheless about the mechanisms at play during the evolution of disease originating in the gut. Our aim is to further characterize our previously established model, exhibiting late PD-like symptoms after repeated gut infection of mice deficient in PTEN-induced kinase 1 (Pink1 KO). We performed single cell transcriptomic analyses of colonic immune cells of Pink1 KO mice following acute bacterial infection as well as in vitro approaches to decipher inflammatory-mediated mechanisms of enteric neuron dysfunction. Our findings underscore that infected Pink1 KO mice display enhanced intestinal inflammation pointing to an aberrant myeloid cell lineage as drivers of early disease. The dysregulation in the innate immune response instigates a pro-inflammatory milieu conducive to enteric neuronal damage, which presumably underlies the gut dysmotility observed in Pink1 KO mice following acute infection. Furrthermore, activated $CD14^+$ monocytes isolated from blood of PD patients also reveal similarities in gene expression signatures promoting inflammation and interleukin-1 signaling as with intestinal Pink1 KO monocytes after acute infection. The reduced PINK1 expression in activated $CD14^+$ monocytes of PD patients may mimic a loss-of-function phenotype as seen with Pink1 KO mice. Collectively, we propose that Pink1 KO mice following acute intestinal bacterial infection constitute an optimal model to investigate neuroimmune-related dysregulation underpinning prodromal PD pathogenesis.

Keyword: *Parkinson's disease, Intestinal inflammation, Infection, Enteric neurons, Single cell sequencing*

#178 TCR-activated MAIT cells promote recovery from CNS inflammation

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In multiple sclerosis (MS), neuroinflammation with ensuing demyelination and neuro-axonal injury is likely initiated by autoreactive T cells. Mucosal-associated invariant T (MAIT) cells represent a highly abundant unconventional T cell subset in humans that recognize with their semi-invariant T cell receptor (TCR) bacterial and yeast antigens derived from riboflavin metabolites presented by the non-classical MHC-related class I molecule, MR1. While MAIT cells infiltrate the central nervous system (CNS) during MS, their involvement in the pathogenesis remains unknown. Here, we discovered an accumulation of MAIT cells in the inflamed CNS of experimental autoimmune encephalomyelitis (EAE) mice. Characterization of MAIT cells in the inflamed CNS revealed that they belong to the MAIT17 (RORγt$^+$) and MAIT1/17 (T-bet$^+$, RORγt$^+$) subsets and show inflammatory as well as tissue-regenerative functions. In addition, MAIT cells in the CNS were strongly activated via cytokines and their TCR as we could show by transcriptome profiling and Nur77GFP reporter mice. Blocking TCR activation with an anti-MR1 antibody resulted in an exacerbated EAE disease course, whereas enhancing TCR activation with the cognate antigen, 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), led to an ameliorated EAE disease course. In summary, our findings indicate that MAIT cells promote recovery from CNS inflammation, likely mediated via their tissue-regenerative functions after TCR activation.

Keyword: *Mucosal-associated invariant T (MAIT) cells, Experimental autoimmune encephalomyelitis (EAE), Multiple sclerosis (MS)*

#179 BASOPHILIC ONCOSTATIN M FUELS NOCICEPTOR NEURON-INDUCED ASTHMA

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Severe asthma affects 4-8% of patients but accounts for 60% of healthcare costs due to the lack of response to, and consequently a high demand for, corticosteroid treatments in these patients (1). Recently published single-cell RNA sequencing predicts that airway-innervating jugular vagal C-fiber neurons expressing oncostatin M (OSM) receptor (OSMR) respond to bronchoconstrictors, histamine, serotonin, and leukotriene C4 (2, 3) and that Osm expression is enriched mouse lung basophils (4). Thus far, we have confirmed the enriched Osm expression in fluorescent-sorted lung basophil by qPCR. More interestingly, such basophilic Osm expression was found higher during ovalbumin-induced allergic airway inflammation. In cultured neurons dissociated from jugular-nodose complex treated with recombinant OSM, we found increased calcium influx in responses to capsaicin, a compound from chili pepper that activates the ion channel transient receptor potential vanilloid 1 (TRPV1), and chloroquine, a Mas-related G-protein coupled receptor member A3 (Mrgpra3)-activating pruritogen, assessed by real-time calcium imaging, suggesting a sensitizing role of OSM in nociceptor and pruritogenic activations in

vagal sensory neurons. Moreover, OSM-treated neurons express higher Trpv1, but not Mrgpra3 mRNA, suggesting that the heightened response to chloroquine is due to the increased TRPV1 expression, but not Mrgpra3 per se. The sensitizing mechanism and pathophysiological roles of this OSM-C-fiber axis in an asthmatic context remain to be unveiled.

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Keyword: *Asthma, Jugular sensory neuron, Oncostatin M, Basophil*

#182 Inhibition of Bruton's tyrosine kinase interferes with meningeal inflammation, microglia activation and subpial cortical demyelination in CNS autoimmunity

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MS is a chronic demyelinating disease of the central nervous system (CNS) that is usually diagnosed as relapsing-remitting in nature (RRMS). Most people who live with RRMS will ultimately develop secondary progressive MS (SPMS) as they age. RRMS can be treated with disease modifying therapies (DMT) targeted at immune cells, and more than a dozen approved therapies are available to choose from. However, most of these therapies fail to alleviate SPMS, suggesting that the pathology of this form of the disease is very different than RRMS. While RRMS is driven by invasion of the CNS by peripheral lymphocytes that breech the blood-brain barrier (BBB), pathological evidence and imaging studies provide evidence that immune cells persist in the SPMS CNS, where they are discretely localized to the leptomeninges adjacent to areas of cortical injury. It is conceivable that the localization of immune cells behind the BBB may limit the ability of immunomodulators to impact immune-associated pathology in progressive MS. Recent efforts have therefore focused on the design of therapies that can cross the BBB to target CNS compartmentalized inflammation. LOU064 is a small molecule that inhibits the BTK (Bruton's tyrosine kinase), a cytoplasmic tyrosine kinase that has been shown to be involved in the regulation of B cell proliferation and activation process. We tested the impact of LOU064 treatment in an experimental autoimmune encephalomyelitis (EAE) adoptive transfer mouse model for MS. Our results show that the treatment of EAE mice with LOU064 induces a reduction of the B and T cells in the meninges, less macrophages/microglia cells in different areas of the brain, as well as less demyelination

in the parenchyma of treated mice compared to the control group that received only the vehicle In conclusion, our data suggest that LOU064 is a promising molecule to consider for the treatment in the EAE mouse model of MS.

#186 TSG101, a novel marker of highly phagocytic disease-associated microglia in the Alzheimer's disease brain

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Background: Different microglial populations have been described in the Alzheimer's disease (AD) brain, including microglia that polarize into either a neurotoxic or a neuroprotective phenotype, termed Disease-associated microglia (DAM). Here, we identified and characterized a sub-population of microglia with high phagocytic activity of Amyloid beta (A-beta) which appears in the adult 5xFAD mouse brain.

Methods: CD11b+ microglia were extracted from 7 months old 5xFAD mice and activated with LPS to mimic the inflammatory environment of the AD brain ex-vivo. Using FACS sorter we separated microglia with high phagocytic activity of A-beta and then compared their proteome to microglia with low or negative A-beta phagocytic activity. Correlation to other known microglial sub-population was performed by image analysis of additional microglial markers in vivo and in vitro.

Results: Comparing the proteomics in microglia with high versus low/negative A-beta phagocytic activity pointed at several proteins that identify the high A-beta phagocytic microglia. Specifically, high A-beta phagocytic microglia showed increased expression of Tumor susceptibility gene 101 (TSG101), a member of the endosomal sorting complexes required for transport (ESCRT) machinery, and responsible for cargo delivery destined for degradation in lysosomes. TSG101-high microglia also exhibited very high phagocytic activity for Tau and alpha-Synuclein peptides. TSG101-high microglia expressed several DAM markers, such as CD11c, TREM2 and LPL, as indicated by immunofluorescent staining and image analysis. Staining of sequential brain sections from 7 months old 5xFAD mice to examine the association of these cells with Amyloid plaques, showed that TSG101-high, and TREM2+ IBA1+ microglia are localized in peri-plaque areas.

Conclusions: High TSG101 expression is a marker for highly phagocytic microglia and is in good correlation with DAM markers. We suggest TSG101 as a novel DAM marker. The especially high A-beta phagocytic phenotype of TSG101-high DAM supports their central role in A-beta clearance.

Keyword: *microglia, Alzheimer's disease*

#187 Neural Precurso Cells Support of Microglia-Mediated Amyloid Beta Clearance Fails in the Alzheimer's Brain

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Background: Early efforts to remove Amyloid beta (A-beta) by Microglia eventually fail, resulting in increased burden of A-beta deposition in the Alzheimer's disease (AD) brain. Neural precursor cells (NPC) possess powerful immune-modulatory and trophic properties, raising the notion of serving as brain protectors. We hypothesized that resident NPC support microglia in removing A-beta, and that there is acquired failure of AD-brain NPC in supporting microglia-mediated A-beta clearance.

Methods: CD11b+ microglia were extracted from 7 months old 5xFAD mice and activated with LPS ex vivo. Microglia were co-cultured with NPC from either wild type (wt) newborn mice or freshly isolated from 7 months old wt/5xFAD mice, expressing GFP under the Nestin promoter. Microglia phagocytic activity of latex beads or A-beta were measured by image analysis, and gene expression by real-time PCR.

Results: We identified a sub-population of Iba1+ microglia with high A-beta phagocytic activity in brain sections of 7-months old 5xFAD mice and in a phagocytosis assay ex vivo. LPS activation increased latex beads uptake but decreased the fraction of microglia with high A-beta phagocytic activity. Time-lapse microscopy showed that co-culturing wt NPC from newborn mice with 5xFAD microglia increased the fraction of microglia with high A-beta phagocytic activity but had no effect on latex beads uptake. Co-culturing with newborn wt mice NPC induced increased expression of TREM2, TLR2, RAGE and MHC-II in microglia. Freshly isolated sub-ventricular zone Nestin-GFP+ NPC from 7-months old wt mice had a similar trophic effect to increase the fraction of high A-beta phagocytic microglia. However, freshly isolated Nestin-GFP+ NPC from 7-months old 5XFAD mice lost their ability to support microglia. Both wt- and 5XFAD- Nestin-GFP+ NPC from 7-months old mice increased microglial-mediated A-beta degradation by similar measures, with a trend of reduced effect by 5xFAD NPC.

Conclusions: Neural precursor cells increase the expression of microglial receptors involved in A-beta phagocytosis, and the fraction of microglia with high A-beta phagocytic activity. Resident AD brain NPC fail to support A-beta clearance by microglia, leading to accelerated disease pathogenesis.

Keyword: *Neural precursor cells, microglia, Alzheimer's disease*

#199 Myelin-reactive B cells exacerbate the severity of CD4+ T cell-driven CNS autoimmunity in an IL-23-dependent manner

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Background

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) that has traditionally been considered a T cell-mediated disease. However, accumulating evidence points to a crucial role for B cells in disease processes. Experimental autoimmune encephalomyelitis (EAE) is a well-established mouse model for studying the immune aspects of CNS autoimmunity.

Objectives and Methods

In order to study the paramount interaction of B and T lymphocytes in disease processes, we immunized transgenic $IgH^{[MOG]}$ mice with the peptide MOG_{35-55}. $IgH^{[MOG]}$ mice possess a transgenic immunoglobulin heavy chain derived from a monoclonal antibody specific to myelin oligodendrocyte glycoprotein (MOG), a key target for autoimmune responses. This mouse model associated with $MOG_{[35-55]}$ autoantigen that drives an mandatory $CD4^+$ T cell-driven response is a useful model to study B and T cell interactions.

Results

We found that immunized $IgH^{[MOG]}$ mice rapidly developed severe EAE. While the frequency and

absolute number of CNS-infiltrating B cells was similar between WT and IgH$^{[MOG]}$ mice, a greater frequency of class-switched and inflammatory cytokine-positive B cells were seen in the IgH$^{[MOG]}$ CNS. We observed an increased presence of class-switched and inflammatory cytokine-positive B cells in the IgH$^{[MOG]}$ CNS, as well as a greater frequency of IL-17- and GM-CSF-producing CD4^{+} T cells. Production of the Th17 maintenance factor IL-23 was increased from IgH$^{[MOG]}$CNS-infiltrating B cells, and in vivo blockade of IL-23p19 strongly attenuated disease severity in IgH$^{[MOG]}$ mice. Strikingly, we observed an increased frequency of PD-1^{+}CXCR5^{-} T peripheral helper (Tph)-like cells in the CNS of IgH$^{[MOG]}$ mice and we also found that the meninges of immunized IgH$^{[MOG]}$ mice were characterized by an accumulation of tertiary lymphoid organs. Both Tph accumulation in the CNS, as well as meningeal inflammation, were again sharply reduced upon IL-23p19 blockade in vivo. Intriguingly, the expression of IL23a transcript in the cerebrospinal fluid of MS-affected individuals was positively correlated with the frequency of B cells.

Conclusion

Altogether, these data show that MOG-specific B cells contribute to severe CD4^{+} T cell-driven EAE by promoting CNS accumulation of Th17 and Tph cells, as well as tertiary lymphoid organs in the CNS meninges, in a IL-23 dependent manner.

Keyword: *Multiple Sclerosis (MS), Experimental autoimmune encephalomyelitis (EAE), B and T cell interactions, tertiary lymphoid organs (TLOs), T peripheral helper (Tph) cells*

#208 Temporal Transcriptomic and Proteomic Dynamics in the Hippocampus of ZnT3-Mutant mice with Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is a neuroinflammatory disease characterized by progressive neurodegeneration, particularly affecting the hippocampus. Despite the severity of neurodegenerative consequences, underlying mechanisms and treatment options remain elusive. Zinc (Zn), an essential trace element in the brain, plays important roles as enzyme cofactors, in the immune system, and as synaptic neurotransmitters. Zinc transporter 3 (ZnT3) facilitates zinc accumulation in synaptic vesicles. Genetic deletion of ZnT3 leads to a deficit of synaptic zinc throughout the brain. We hypothesized that synaptic Zn affect neuroinflammation and the degenerative process in multiple sclerosis. Here we evaluated RNA-Seq-based transcriptomic and proteomic temporal changes (day 0, peak, 8 week) of the hippocampus after induction of Experimental Autoimmune Encephalomyelitis (EAE) in ZnT3-mutant mice. After EAE induction, clinical scores of EAE were profoundly reduced at peak and 8 weeks in ZnT3$^{-/-}$ mice. In the transcriptomic and proteomic analyses, immune-related and synaptic gene expression strongly and

dynamically changed after EAE induction, while their patterns differed between ZnT3$^{+/+}$ (EAE-WT) and ZnT3$^{-/-}$ (EAE-KO). In the EAE-WT group, both immune-related mRNA and proteins exhibited the same directional change, peaking at the highest level and decreasing by the 8-week point. Conversely, synaptic-related mRNA and proteins showed an opposite pattern, reaching their lowest level at the peak and increasing beyond the peak at 8 weeks. In the EAE-KO group, immune-related mRNA and proteins followed a similar trend over time, but with intermediate levels at the peak and the highest levels at 8 weeks. Notably, the upregulation of immune-related proteins was modest at the peak and 8-week point, significantly lower than the peak level observed in EAE-WT mice but higher than the 8-week level in EAE-WT mice. Taken together, these findings suggest that synaptic Zn affect immune and synaptic-related transcriptomic and proteomic changes in the EAE mouse, but its roles vary depending on the time point following the induction of inflammation.

Keyword: *EAE, multiple sclerosis, zinc, ZnT3, MS*

#259 ASH41020, A Novel Hydroxyl Dendrimer CSF1R Tyrosine Kinase Inhibitor 'Dendranib' Nanomedicine, Polarizes Macrophages Toward an Anti-Inflammatory Phenotype and Improves Disease Severity in a Mouse Model of Multiple Sclerosis

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Multiple sclerosis (MS) is an inflammatory disorder of the central nervous system (CNS) whose pathogenesis is mediated in part by infiltrating monocytes and macrophages. Colony-stimulating factor-1 receptor (CSF1R) is a receptor tyrosine kinase critical for survival and proliferation of CNS microglia, peripheral tissue macrophages and blood myeloid cells. Targeting CSF1R with selective tyrosine kinase inhibitors such as dasatinib and masitinib decreases experimental autoimmune encephalomyelitis (EAE) severity and delays disease onset.

ASH41020 is a new 'dendranib' nanomedicine that selectively inhibits CSF1R tyrosine kinase only in activated microglia and macrophages. Effects of ASH41020 on macrophage phenotype (M1 vs. M2) were studied in vitro in differentiated human primary monocytes. Effects of ASH41020 on macrophage phenotype and clinical symptoms were also studied in a mouse EAE model of MS. Symptomatic animals (EAE mean score of ~1.5) were treated for 14 days at 20, 60 or 200 mg/kg ASH41020 IP daily or every other day and compared to fingolimod (3 mg/kg, PO daily) as a positive control. Clinical scores were monitored daily, and spinal cords were analyzed for M1 and M2 macrophage markers using flow cytometry.

ASH41020 increased the percentage of M2 macrophages in M1 and M2 polarized cell cultures and increased number of M2 macrophages without affecting M1 macrophages in the spinal cord of EAE mice (200 mg/kg daily dose). Importantly, daily administration of ASH41020 also significantly reduced EAE disease severity compared to vehicle control at all dose levels tested. The magnitude of this effect was similar to that of fingolimod.

These data demonstrate that ASH41020 directs macrophage polarization toward the anti-inflammatory M2 phenotype, which in turn ameliorates symptoms in a mouse model of MS. These preclinical studies suggest that ASH40120 is a potent anti-inflammatory and immunomodulatory agent that warrants further

development as a promising treatment for MS patients.

Keyword: *Multiple Sclerosis, Inflammation, Macrophages, Microglia, Immunomodulation*

#267 SRSF3 modulates immune response in the APP/PS1 model of disease

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Growing evidence suggests that chronic deregulation of innate immunity may represent one of the key elements in its pathobiology of Alzheimer's disease (AD) and related neurodegenerative disorders. Rebalancing and/or strengthening the innate immune response has been proposed to be therapeutically relevant in AD. Microglia are the principal immune cells of the brain, and once activated, they can acquire a wide repertoire of immune profiles ranging from the classical pro to anti-inflammatory phenotypes. Its apparent profile shift is particularly evident in the context of chronic neurodegeneration. We recently described a novel ribosome-based regulatory mechanism orchestrated by the RNA binding protein (RBP) SRSF3. It controls innate immune gene translation in acutely activated microglia. SRSF3 is the smallest member of the serine/arginine RBP family involving in a wide array of biological processes in health and disease. Here, we aim to investigate the impact of SRSF3 as a regulator of innate immune response of activated microglia in AD. Brains of APP/PS1, presymptomatic and symptomatic mice were used to assess the expression level of amyloid beta (Abeta) peptides and disease-associated markers like TREM2, CD33, LILRB4a. WT age-matched littermates were used as controls. The levels of the active form of SRSF3 (pSRSF3) were also evaluated throughout the disease. Then, following the assessment of the disease, 1 year old APP/PS1 mice were treated intranasally with anti-SRSF3 antisense morpholino to reduce its level expression. The levels of pSRSF3/SRSF3, Abeta peptides, phagocytic, pro-inflammatory and neuronal markers were evaluated by western blot analysis while mice cognition was assessed by Passive Avoidance Task (PAT) and Novel Object Recognition (NOR) behavioural tests. With the progression of the disease, we observed an increase of Abeta peptides and pSRSF3/SRSF3 levels whereas the expression level of TLR2, TREM2 and LILRB4a did not change. Interestingly, treatment with anti-SRSF3-Morpho decreased the levels of Abeta peptides and increased the expression levels of microglial markers (TLR2, LILRB4a and TREM2) and the neuronal markers (synaptophysin and PSD95) in comparison to non-treated mice. In the PAT and NOR tests, recognition memory was improved by the treatment when compared to non-treated mice. Our results revealed that SRSF3 regulates expression patterns of targeted immune genes suggesting its immune-modulatory potential in AD-like pathologies.

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Keyword: *Microglia, Innate Immune Response, Neurodegeneration, Antisense oligonucleotides*

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#268 Eculizumab in AQP4+ Neuromyelitis Optica Spectrum Disorder: 3 Years of Data From Japanese Postmarketing Surveillance

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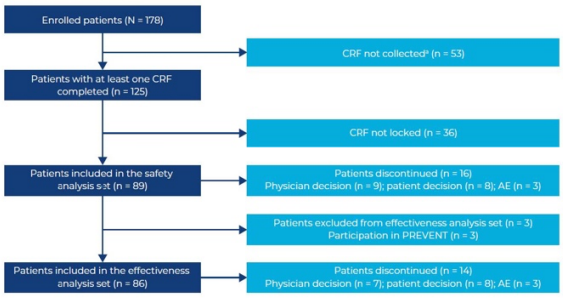
Background and aims: Eculizumab is approved in Japan for prevention of aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder (AQP4+ NMOSD) relapse and is undergoing mandatory post-marketing surveillance (PMS) of real-world use.

Methods: This PMS interim analysis assessed long-term safety and effectiveness of eculizumab in patients in Japan from approval (November 2019) to data cut-off (October 2022).

Results: The safety set comprised 89/178 patients; 16 discontinued (9 physician decisions, 8 patient decisions, 3 adverse events [AEs]). Overall, 62 AEs (25 deemed treatment-related) were reported in 31 patients; of the 62 AEs, 40 were serious AEs (14 deemed treatment-related) occurring in 22 patients. No meningococcal infections occurred. The effectiveness set comprised 86 patients. In the 2 years (172.00 patient-years [PY]) before eculizumab treatment, the relapse rate was 0.68/PY; 28 patients (32.6%) had 1 relapse and 33 patients (38.4%) had ≥2 relapses. During eculizumab treatment (89.42 PY), relapse rate was 0.01/PY (1 relapse). During the 6 months before eculizumab treatment, 46 patients (53.5%) were receiving immunosuppressant therapy (IST), whereas during the 6–12 months after eculizumab, 25 (44.6%) were receiving IST. The proportion of patients receiving prednisolone >10 mg/day decreased from 44.2% at 24–20 weeks before eculizumab treatment to 18.2% and 11.2% at 52–56 and 100–104 weeks after eculizumab, respectively.

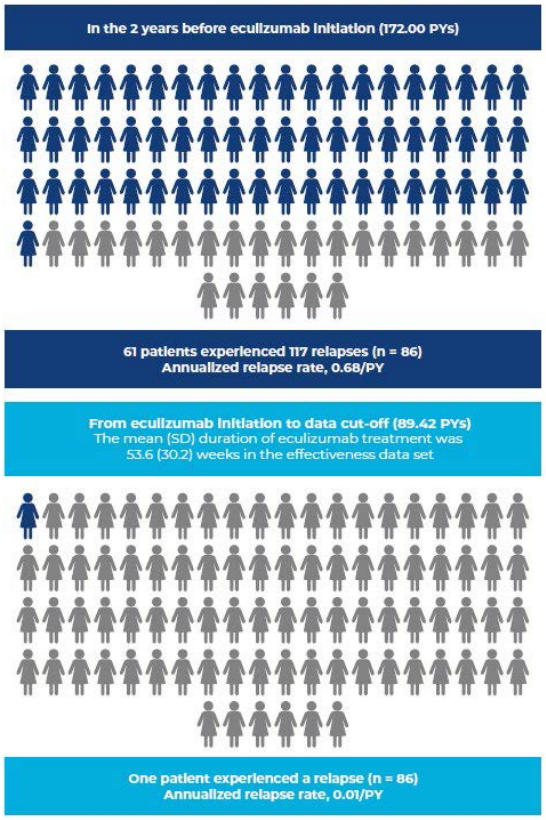
Conclusions: In a real-world setting, eculizumab was highly effective in preventing relapses and well-tolerated in patients in Japan with AQP4+ NMOSD, consistent with findings from the PREVENT study. The observed reduction in IST use, also aligned with other real-world experiences, underlines the benefits of C5 inhibition in these patients.

Figure 1. Patient disposition



[a]Patients for whom a CRF was not collected were not included in the analysis.
Patients may be counted to more than one reason for discontinuation.
AE, adverse event; CRF, case report form.

Figure 2. Incidence of relapses before and after eculizumab initiation



PY, patient-year; SD, standard deviation.

Table 1. Summary of patients with treatment-related AEs

Event, n (%)	Safety analysis set (n = 89)
Any treatment-related AE	15 (16.9)
Any treatment-related SAE	8 (9.0)
Gonococcal infection	2 (2.2)
Bacteraemia	1 (1.1)
Cellulitis	1 (1.1)
Meningitis bacterial	1 (1.1)
Meningitis herpes	1 (1.1)
Pneumonia	1 (1.1)
Bacterial sepsis	1 (1.1)
Device related infection	1 (1.1)
Pulmonary hypertension	1 (1.1)
Systemic lupus erythematosus	1 (1.1)
Cystitis haemorrhagic	1 (1.1)
Renal impairment	1 (1.1)
Pyrexia	1 (1.1)

AE, adverse event; SAE, serious adverse event.

#271 Mice with a Parkinson's Disease-Associated Mutation in LRRK2 have Altered Early Response to Intestinal Infection

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The gut-microbe-brain axis is an area of intense interest in Parkinson's disease (PD) research. Our previous data demonstrated that infection of mice with deletion of the PD- associated gene Pink1 triggered the development of motor symptoms later in life. While the overall course of infection was similar in WT and Pink knockout mice, we showed that Pink1 modulates the host response to intestinal infection. We are now evaluating whether other PD-associated genes are also implicated in early intestinal immune responses, such as the leucine-rich repeat kinase 2 (LRRK2). To this end, male and female wild-type (WT) and LRRK2 knock-in Gly2019Ser (KI) and knockout (KO) mice were infected with the mouse intestinal pathogen Citrobacter rodentium and immune response was evaluated. Our results revealed that, at day 7 of infection, KI mice control better the infection compared to the WT and KO mice, evaluated by the CFU counts. Lipocalin-2 levels in feces were increased in KI male mice and equal in KO and WT mice, measured by ELISA. Flow cytometry analysis showed that the C. rodentium infection in mice induced MHC-II expression in epithelial cells in KI males after infection. In colonic lamina propria (LP), infected KI mice had increased neutrophils and B cells, while a decrease was seen in macrophages and dendritic cells, compared to WT and KO mice. RT-qPCR of cytokines in colonic LP cells showed an increase in IL-17a and curiously, a decrease in IL-1 beta in KI mice compared to WT mice. Accordingly, scRNA sequencing demonstrated that neutrophils and B cells were the cells that harboured the greatest differences in gene expression, with a variety of biological processes upregulated in neutrophils and suppressed in B cells. Our results suggest that the LRRK2 G2019S mutation modulates the

intestinal immune response to bacterial infection.

#282 Senescent-like phenotype in neurons in multiple sclerosis

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Background: Multiple sclerosis (MS) is a complex neuroimmune disease. Loss of brain volume, particularly of the grey matter, is one of the best clinical correlates to sustained disability for MS patients, suggesting that neurodegeneration in MS may be a relevant area of study in order to identify novel therapeutics. However, in order to identify neuroprotective drugs, it is essential to understand how neurons are altered by injurious inflammation.

Objectives: Identify novel dysregulated pathways in neurons in experimental autoimmune encephalomyelitis (EAE) and MS through multimodal sequencing and identify potential ways to protect neurons in the disease.

Methods: RNA-sequencing, miRNA-sequencing, and ATAC-sequencing was performed on retinal ganglion cells from EAE mice at different timepoints and underwent differential gene and pathway analysis. Additionally, previously published single-nucleus sequencing datasets from neurons from MS patients were reanalyzed to validate findings in EAE. Immunohistochemistry (IHC) analyses were conducted to validate dysregulation of pathways of interest. Visual acuity was measured by the optomotor response in EAE animals treated with drugs intended to target dysregulated pathways.

Results: Pathway analysis of RNA-sequencing from EAE mice suggested a preponderance of senescence-associated pathways in neurons and correlated with transcriptomic changes seen in naïve aged mice. Equally, single-nucleus sequencing of cortical neurons from grey matter of MS patients similarly confirmed the presence of a senescence like signature, characterized by alterations to cell cycle pathways and accumulation of DNA damage pathways. IHC confirmed that retinal ganglion cells in EAE mice accumulate yH2AX in their nuclei, and equally found increased expression of cell cycle proteins, and changes to histone modification marks. **Conclusions:** Inflamed and injured neurons undergo changes that are reminiscent of a senescence-signature, predominantly with alterations to cell cycle related proteins and DNA damage pathways. Drugs targeting senescence in neurons may be useful to preserve neuronal function over time, and is being investigated in EAE.

Keyword: *neuron, multiple sclerosis, aging, senescence, sequencing*

#298 Contribution of inflammation to the underlying pathobiological mechanisms of back pain

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Degeneration of the intervertebral discs in humans can lead to several pathologies associated with back pain including myelopathy and radiculopathy. Degenerated discs exhibit ossification which can injure the spinal cord in

myelopathy or the nerve roots in radiculopathy. A subset of patients can be relieved by surgical interventions, but additional therapeutic options are greatly needed. The underlying mechanisms implicated in these different spinal disorders are poorly understood, thus impeding the development of relevant therapeutics. Notably, degenerated discs show enhanced nerve innervation, neovascularization, and infiltration of peripheral immune cells. Whether specific inflammatory mediators participate in degenerated disc pathologies remains unclear.

We hypothesize that biomarkers in peripheral blood can identify defective repair and/or enhanced inflammation pathways involved in the pathobiological mechanisms of different forms of spinal pathologies.

Plasma and peripheral blood mononuclear cells (PBMCs) were collected from patients (n = 80) just prior to their spinal surgery. Levels of inflammatory cytokines, chemokines, and growth factors were assessed by ELISA multiplex and SIMOA assays and compared with samples from sex/age-matched healthy controls (HC, n = 26). Clinical data collected from patient's medical records include age, sex, pain measurements, and imaging data (i.e. MODIC Score, narrow canal, and nerve damage).

We observed that patients with myelopathy exhibit significantly elevated plasma levels of neurofilament (NFL) compared with HC or patients with radiculopathy. Notably, NFL levels are higher in patients with a narrow spinal canal than those with a normal canal. Moreover, plasma levels of inflammatory markers CRP, sICAM-1 and TNF are significantly increased in myelopathy patients compared with HC. In contrast, CCL22 is higher in plasma samples from radiculopathy patients than HC. Finally, plasma levels of EGF, a growth factor, are significantly reduced in patients with either myelopathy or radiculopathy compared with HC.

Our results suggest that the signature of blood biomarkers including both inflammatory markers and growth factors could stratify patients with degenerated disc-associated pathologies. The identification of altered molecular and cellular signatures in patients with degenerated disc pathologies will guide investigations on pathobiological mechanisms and the development of additional therapies.

#308 Role of transcription factor Irf3 in coordinating microglia activity in demyelinating lesions of the brain

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Introduction:
Multiple Sclerosis (MS) is a degenerative auto-immune disease of the central nervous system (CNS) associated with myelin and axonal damage. As tissue-macrophage of the brain, microglia are important effectors of enhanced inflammatory response in MS, but how these cells are regulators is not fully understood. Previous studies by others and from our group indicated that that microglia associated with myelin damage upregulate an important number of antiviral inflammatory genes. Furthermore, our previous analysis of distal regulating elements suggested an important contribution of transcription factor Irf3 in mediating this transcriptional signature.

Hypothesis: Irf3 regulates neuroinflammatory responses and neuropathologies associated with brain demyelinating lesions induced by cuprizone diet.

Methodology and Results: Two series of experiments were performed to test this hypothesis.
In a first series of experiments, we assessed role of Irf3 peak accumulation of neuropathologies. For this, adult male wild-type (WT) mice and Irf3 knock-out (Irf3 KO) mice underwent cuprizone diet for 5 weeks, and were then sacrificed for histology and microscopy analyses. Results revealed similar levels of demyelination over the corpus callosum and the external capsule brain areas between the two, as measured by modified Gallyas staining. However, absence of Irf3 increased significantly IBA1 positive staining distribution over the external capsule and deep layers of the cortex.
In a second series of experiments, we analyzed the role of Irf3 during the remyelination process that occurs after termination of the cuprizone diet. For this, WT and Irf3 KO mice were fed cuprizone for 5 weeks, after which they were returned to regular/normal diet for 7 days, and then sacrificed for histology and microscopy analyses. Here, absence of Irf3 led to a significantly weaker intensity of Gallyas staining, which was also accompanied by a significantly elevated IBA1 staining distribution over the external capsule and deep layers of the cortex.

Conclusion: Thes data show that Irf3 activity, at the tissue level, limits microglial inflammatory activity and/or microglial accumulation at lesioned areas. This in turn appear to interfere with repair processes. Current experiments are examining whether suppressing Irf3 specifically in microglia is sufficient for these defects to arise.

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Keyword: *Microglia, Cuprizone, Multiple Sclerosis, Irf3*

#314 The function of CD11c+ B cells in peripheral blood mononuclear cells in multiple sclerosis

Victoria Hyslop Hvalkof[1], Sahla El Mahdaoui[1], Marie Mathilde Hansen[1], Rikke Holm Hansen[1], Jeppe Romme Christensen[1], Finn Sellebjerg[1,2], Marina Rode von Essen[1]

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Introduction:
Multiple sclerosis (MS) is an immune-mediated neurologic disease causing demyelination and axonal damage. B cells are implicated in the pathogenesis, emphasized by the high efficacy of anti-CD20 monoclonal antibody therapy. B cells are a highly heterogeneous population, and it is yet unclear which B cell subsets are involved in MS pathogenesis. $CD11c^+$ B cells is a subset that are expanded during and following viral infections and in autoimmune diseases. $CD11c^+$ B cells has also been proposed as a mediator of autoimmune disease and a possible link between Epstein-Barr virus infection and MS. In MS, $CD11c^+$ B cells were more prevalent in cerebrospinal fluid compared to paired peripheral blood samples, which is also seen in a MS mouse model. However, the function of $CD11c^+$ B cells in MS is yet unknown.

Objectives/Aim:
To study the function of $CD11c^+$ B cells in healthy controls (HC) and in persons with relapsing remitting MS (RRMS).

Methods:
Peripheral blood mononuclear cells (PBMC) were cultured from 12 treatment-naïve persons with

RRMS and 12 age and sex matched HC. The PBMC cultures were stimulated with a Toll-like receptor 9 (TLR9) agonist for 48h follow by a brief stimulation with PMA/ionomycin for 4h. Intracellular cytokine production of $CD11c^+$ and $CD11c^-$ B cells were then analyzed by flow cytometry.

Results:

We found a higher frequency of $CD11c^+$ B cells in TLR9-stimulated PBMC cultures in HC (fig. 1, median 9% compared to 15%, $p<0.001$) and RRMS (fig. 1, median 11% compared to 19%, $p<0.001$).

The frequencies of Interleukin (IL)-10, Transforming Growth Factor (TGF)-beta and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) producing cells were higher in $CD11c^+$ than $CD11c^-$ B cells in RRMS and HC (fig.2DEF, $p<0.001$). In contrast, we found lower frequencies of $CD11c^+$ B cells producing IL-6 in HC and RRMS (fig. 2C, $p<0.001$), and Tumor Necrosis Factor (TNF)-alpha in RRMS (fig. 2A, $p<0.05$) compared to $CD11c^-$ B cells. We also found a higher frequency of IL-6-producing $CD11c^-$ B cells in RRMS compared to HC (fig. 2C, $p<0.05$).

Conclusion:

This study shows an induction of $CD11c^+$ expression on B cells upon TLR9 stimulation. It demonstrates a functional difference between $CD11c^+$ and $CD11c^-$ B cells, and that $CD11c^+$ B cells have the potential to contribute to a pro-inflammatory and regulatory response. Additional studies with higher power are required, though the results of this study indicate that the cytokine production of $CD11c^+$ B cells in RRMS is altered.

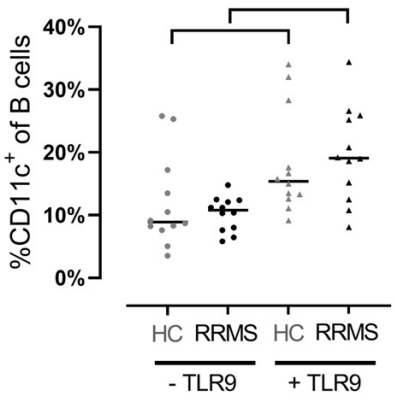

Figure 1: Frequency of CD11c+ B cells without (dots) and with (triangles) TLR9 stimulation for healthy controls (HC, grey) and relapsing-remitting multiple sclerosis (RRMS). Significant differences are shown with lines. Thick line: p<0.001.

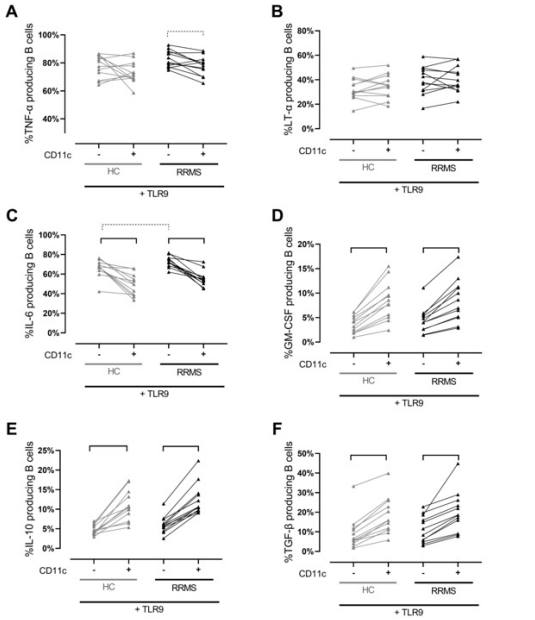

Figure 2: Cytokine production by CD11c- and CD11c+ B cells. Frequency of B cells producing A: tumor necrosis factor alpha, B: lymphotoxin alpha, C: interleukin-6, D: granulocyte-macrophage colony-stimulating factor, E: interleukin-10, F: transforming growth factor beta. Healthy controls (HC, grey) and treatment-naive persons with relapsing-remitting multiple sclerosis (RRMS). Shown for B cells with toll-like receptor 9 (TLR9) stimulation. Significant differences are shown with lines. Dashed line: p<0.05, thick line: p<0.001.

Keyword: *CD11c, B cells, Cytokine, Multiple sclerosis*

#321 Total PLCG2 ablation impairs microglial responses and confers diverse transcriptional alterations in a murine model of Alzheimer's disease

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Many of the genes associated with altered risk for Alzheimer's disease (AD) are predominantly expressed in microglia and affect innate immune responses. Among these genes is phospholipase C gamma 2 (PLCG2), a critical mediator of transmembrane signaling that acts downstream of many immune receptors on microglia, including TREM2. PLCG2 is robustly induced by amyloid pathology in AD and recent transcriptomic studies suggest a vital role for PLCG2 in the immune response to AD pathology, learning, and metabolism. Reduction in PLCG2 activity is associated with exacerbated AD pathology, but the mechanisms underlying these effects remain unclear. Therefore, we explored the impact of Plcg2 ablation or haploinsufficiency on amyloid pathology and microglial response in the amyloidogenic 5xFAD murine model of AD and compared this to 5xFAD mice deficient in Trem2 to establish contributions of upstream signaling. While Plcg2 haploinsufficiency increased X34+ and 6E10+ amyloid plaque pathology, loss of Plcg2 in 5xFAD mice results in similar plaque burden as wildtype and Trem2-deficient mice. Additionally, Plcg2 deficiency significantly impaired microglial interactions with plaques and showed reduced immunoreactivity of microglia activation marker CD68 when compared to Plcg2$^{+/-}$ mice. Transcriptomic analysis revealed several biological processes altered by loss of Plcg2, including pathways associated with the microglial response, metabolism, synapses, and cell signaling. Weighted gene correlation network analysis (WGCNA) produced many significant modules of co-expressed genes such as those associated with immunity, metabolism, mitochondrial respiration, and synaptic connectivity. Importantly, one module was differentially expressed between each genotype and contained many immune-related genes, including disease-associated microglia (DAM) genes. These findings suggest PLCG2 depletion impairs the ability of microglia to effectively transduce surface receptor signals in response to amyloid plaques, leading to a stunted immune response. Overall, this study highlights the importance of PLCG2 in the innate immune response to amyloid pathology and reveals several novel pathways which may be regulated by PLCG2.

Keyword: *microglia, alzheimer's disease, trem2*

#334 Investigating the Impact of Parkinson's Disease-Associated Genes on Intestinal Homeostasis

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There is increasing evidence that Parkinson's Disease (PD) involves gastrointestinal dysfunction. People with inflammatory bowel disease are more likely to develop PD, and a

positive correlation exists between gastrointestinal infection and PD incidence. Furthermore, intestinal symptoms like constipation are commonly observed years prior to motor dysfunction development in people with PD. Intestinal epithelial cells (IECs) provide a physical barrier between luminal contents and host tissue. Loss of barrier function can cause local and systemic inflammation. Our group recently developed a model to investigate the role of the gut in PD, demonstrating that mice with genetic ablation of the PD gene Pink1 exhibited motor phenotypes only when previously infected with a gram-negative intestinal pathogen. Infection in Pink1 KO mice induced presentation of mitochondrial peptides on MHC I in innate immune cells, with establishment of anti-mitochondrial T cells in the periphery and brain. As Pink1 and other PD-associated genes are expressed in IECs, we hypothesize that PD gene mutations may also directly affect the epithelium, their inflammatory response, and ultimately impact early PD pathophysiology. To understand the effects of PD mutations in IECs, we performed single-cell RNA sequencing of IECs isolated from Pink1 KO and WT mice at steady state and following in vivo infection. Our data revealed that PINK1 loss-of-function profoundly affected the intestinal stem cell compartment, transit amplifying cells, and enterocyte lineages following infection. To validate hits from our scRNAseq dataset and determine how PINK1 loss-of-function affects the inflammatory response of the epithelium, we are currently using ex vivo colonic organoids treated with lipopolysaccharide (LPS) – a component of gram-negative bacteria. To investigate epithelial-immune cross talk, our preliminary data has demonstrated that CMT93 mouse rectal epithelial cells treated with IFN-γ and LPS can present self-mitochondrial antigen to an anti-mitochondrial CD8 T cell line, resulting in their activation. This indicates that the epithelium is directly capable of presenting self-mitochondrial peptides to CD8 T cells under inflammatory conditions. Further validation will be completed in epithelial cells isolated from Pink1 WT and KO mice. By investigating the role of PD genes in the gastrointestinal tract, these studies carry important implications for understanding PD initiation and progression.

Keyword: *Parkinson's Disease, Intestinal infection, Organoids, Intestinal epithelial cells*

#351 Characterizing neuronal PAS-domain containing protein 4 expression in inflammatory and neurodegenerative settings relevant to multiple sclerosis

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Neurodegeneration in MS is associated with inflammatory cells and their mediators, which alter neuronal calcium homeostasis. Neuronal PAS-domain containing protein 4 (NPAS4) is a neuronal transcription factor activated by intracellular calcium shifts that regulates genes involved in inhibitory synapse development, plasticity and survival. We showed CNS NPAS4 expression is altered in inflammatory degenerative settings[1]. NPAS4 RNA increases at early signs of immune cell infiltration but decreases at peak disease in the preclinical MS model experimental autoimmune encephalomyelitis (EAE). To elucidate the neuroprotective properties of NPAS4, we characterized NPAS4 expression in response to inflammatory mediators associated with MS onset and progression.

We assessed NPAS4 protein expression in vivo at different stages of MOG35-55 chronic active EAE and in vitro in mouse neuronally-enriched cortical cultures treated with glutamate (1-30 uM), H_2O_2

(12.5-100 uM) and resting or activated CD4+ T-cells up to 9 hrs. We examined associated changes in the nuclear mean fluorescent intensity (MFI) in NeuN+ cells and percentage of NeuN+NPAS4+ cells.

Basal NPAS4 expression in neuronal cultures at day 10 is negligible in over 90% of NeuN+ cells. NPAS4 protein levels were rapidly increased by 10-30 uM glutamate, with expression intensity the highest at 3 hr of all doses tested; levels returned to baseline by 7-9 hrs. NPAS4 induction was heterogeneous, and maximum induction was observed with 10 uM for 1 hr with 70% positivity compared to 9% in controls ($p<0.0001$). Alternatively, oxidative stress (H_2O_2 50 & 100 uM) increased NPAS4 expression at later exposure points, increasing 3.6-fold compared to controls, which was higher than lower doses ($p=0.003$). Activated, but not resting, CD4+ T cells increase neuronal NPAS4 protein, with maximal increase in MFI at 2 hrs co-incubation. Preliminary analyses in chronic EAE tended to show more cortical NPAS4+NeuN+ cells from onset through late disease (day 54, $p=0.0003$) and may result from diffuse microglial activation and associated oxidative stress.

MS-related inflammatory mediators alter neuronal NPAS4 in vitro, and could explain changes observed in chronic MS models. NPAS4 may serve as a sensitive marker of inflammatory stress in the CNS. Further studies are needed to address the impact of sustained Npas4 on neuronal function and viability and Npas4's potential as a target to limit neurodegeneration in MS.

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Keyword: *neuroprotection, neuroinflammation, neurodegeneration, experimental autoimmune encephalomyelitis, multiple sclerosis*

#363 A tissue engineering approach to evaluate the role of the brain-periphery axis in Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder estimated to affect 5% of the population over age 85. PD is characterized by a loss of dopaminergic neurons in the substantia nigra of the brain, leading to the onset of PD-related motor symptoms such as tremors. Neuropathological hallmarks of the disease include the formation of Lewy bodies, which are large protein inclusions consisting of misfolded alpha-synuclein (aSyn). Mutations in the SNCA gene that codes for the aSyn protein is associated with fully penetrant familial forms of the disease. One of these mutations is the triplication of the SNCA locus resulting in early onset of PD and rapid disease progression compared to idiopathic cases. Braak staging of synucleinopathy propagation proposes that anatomical sites of disease onset could be outside of the central nervous system and may involve the periphery. Furthermore, studies by us and others identified potential defects in the cerebrovasculature, and a compromised blood-brain barrier (BBB) may contribute to PD progression. Based on these observations, we hypothesize that PD progression could be associated with an abnormal infiltration of circulating factors into the brain that is facilitated by BBB dysfunction. Our objectives are to model the PD BBB in vitro

to enable the deep characterization of periphery-brain interactions as a potential trigger of disease onset and propagation. We therefore developed a 3D microfluidic model of the BBB using induced pluripotent stem cells (iPSCs) originating from healthy donors or people with PD harboring the SNCA triplication mutation. These iPSCs are differentiated into cell types of the neurovascular unit, plated into the BBB-chip device, and changes to vascular function under various experimental conditions can be measured. In parallel, BBB organoids are produced to complement the BBB-chip model and perform advanced cellular and molecular biology experiments. Our results show barrier deficits in the pathological SNCA condition, characterized by increased infiltration of peripheral proteins (e.g. IgG, albumin and aSyn monomers), elevated secretion of pro-inflammatory cytokines, and neurodegeneration. In this context, it appears that SNCA triplication induces the formation of a dysfunctional barrier, which would promote the entry of potentially neurotoxic circulating proteins from the periphery to the PD brain.

#391 Amyotrophic lateral sclerosis is associated with altered peripheral neutrophils

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressing neurodegenerative disease characterized by death of upper and lower motor neurons, causing progressive paralysis. Death occurs 2-4 years following onset, and few treatment options exist. Previous studies have demonstrated that immune dysregulation occurs in ALS, and manipulation of the immune system can increase survival in mouse models of disease. In our own studies, we have shown that peripheral neutrophil numbers are elevated in ALS, and increases in cell numbers are associated with more rapid disease progression and reduced survival.

To further evaluate the role of neutrophils in ALS, ALS patients and healthy controls were recruited to donate blood for immunophenotyping via flow cytometry. Using ALSFRS-R scores collected the same day as the blood draw, statistical analyses were performed to determine whether specific neutrophil activation markers are associated with disease progression. In addition, a second cohort of patients and controls were recruited for a more comprehensive analysis of neutrophil heterogeneity using multidimensional reduction of a comprehensive flow cytometry panel to identify dysregulated populations of neutrophils in ALS.

We found that CD11a, CD11b, CD38, and CD62L expression was elevated on neutrophils analyzed as a whole in ALS patients, and total neutrophil numbers and CD38 expression were key variables in prediction models for diagnosis and progression. Moreover, multidimensional reduction demonstrated differences in neutrophil populations between ALS patients and healthy controls, including both populations unique to ALS and downregulated in ALS compared to controls. The differences in neutrophil activation and overall populations provide compelling evidence that neutrophils play a role in ALS progression, as well as provide novel targets for therapeutics.

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Keyword: *amyotrophic lateral sclerosis, ALS, neutrophil, innate immunity, peripheral immunity*

#393 Effect of cladribine on plasma cells

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Introduction. Cladribine is a nucleoside analogue immune-depleting, central nervous system (CNS) penetrant, disease modifying treatment for relapsing multiple sclerosis. Cell-cytotoxicity is controlled largely by the activity of deoxycytidine kinase (DCK), which is countered by 5' cytoplasmic nucleotidases and to a small extent by adenosine deaminase (ADA). Cladribine can reduce oligoclonal immunoglobulin bands in some individuals with multiple sclerosis. It was hypothesized that cladribine may act on B and antibody-secreting cells within the CNS.

Method. The distribution of DCK and other enzymes was assessed in public mRNA expression data and importantly following the in vitro differentiation of long-lived plasma cells from memory B cells, using growth factors, cytokines, and costimulation. These cultures were used to assess cladribine cytotoxicity of plasma cells.

Results. The mRNA expression in B cell subsets indicated that long-lived plasma cells exhibited lower expression of DCK and elevated ADA than observed in naive and memory B cells. 5' nucleotidases were weakly expressed. This could be replicated during the in vitro differentiation of activated B cells into plasmablasts and plasma cells. Plasma cells were significantly less sensitive to cladribine than cells during the activated B cell-plasmablast or plasmablast-plasma cell transitions in vitro. This indicated limited plasma cell cytotoxicity at physiological doses of cladribine.

Conclusion. Plasma cells are relatively insensitive to the action of cladribine, consistent with the persistence of past-infection immunity and vaccination responses observed, which is an important safety feature for this agent. This suggests that rather than direct induction of apoptosis of plasma cells, any impact on oligoclonal immunoglobulins in the CNS is probably due to targeting of plasma cell precursors and cells supporting plasma cell survival.

#395 Enhanced Th1 and Th17 cell differentiation and function in the absence of Nox2 in EAE

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Enhanced Th1 and Th17 cell differentiation and function in the absence of Nox2 in EAE
Chronic granulomatous disease (CGD) inherits abnormal function of nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase 2 (Nox2). Nox2 originally identified in phagocytes is responsible for the production of reactive oxygen species (ROS) that kill en- gulfed pathogens. Thus, CGD patients are susceptible to infections by bacteria and fungi and also suffer from persistent inflammatory responses in liver, kidney, colon and lung. There have been studies to reveal the function of Nox2 in hyper-inflammatory diseases, especially in multiple sclerosis(MS), but the exact role of Nox2 in MS is still unclear and controversial. To determine whether Nox2 deficiency affects MS, we utilized experimental autoimmune encephalomyelitis models to clarify the recent debates. Furthermore, we attempted to reveal the precise mechanism of Nox2 deficiency in a T cell intrinsic manner by using in vitro activation. MS phenotypes were analyzed in response to myelin-induced experimental model. To understand the underlying mechanisms of exaggerated Th1 and Th17 effector functions, we investigated the degree of T cell activation, levels of activation induced cell death (AICD), and regulatory T (Treg) cell differentiation in Nox2-deficient T cells. All experimental EAE phenotypes including clinical scoring and interferon gamma and interleukin-17a production in spleen were increased through enhanced Th1 and Th17 differentiation in Nox2- null mice. Nox2-deficient T cells also showed diminished Treg cell differentiation through increased AKT phosphorylation and enhanced mito- chondrial ROS production. Our findings indicate that Nox2 deficiency results in exaggerated experimental encephalomyelitis, which is caused by enhanced Th1 and Th17 effector function in a T cell intrinsic manner.

Keyword: *Multiple sclerosis, NADPH Oxidase2, experimental autoimmune encephalomyelitis*

#398 Interferon stimulated genes disrupt neuronal protein and mitochondrial turnover

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Neuron and axon loss are the strongest corrrelates of disease progression in MS. However, the mechanism that contribute to neuron loss following demylination remain unclear. Indeed, axon survival has been noted in many models of chemical demyelination. We recently reported that neurons upregulate interferon stimulated genes--including ISG15--during demyelination. ISG15 is a small ubiquitin-like molecule that can be covalently linked to lysine residues on target substrates through coordination of E1, E2, and E3 ligases. Additionally, ISG15 is capable of "capping" ubiquitin and preventing the formation of polyubiquitination chains. Therefore, we sought to determine whether interferon treatment or ISG15 induction could disrupt ubiquitin-dependent protein and mitochondrial turnover using live cell imaging and fluorescent reporters in primary murine cortical neurons and human IPSC derived neurons. We used degradation signal tagged mCherry (DD-mCherry) and measured degradation kinetics for assement of ubiquitin-proteasomal activity. We used mCherry-GFP-FIS1 (mito-QC) and measured pH dependent quenching of GFP signal (non-colocalized mCherry) as an indicator of mitophagy. We found that interferon treated neurons exhibited slower turnover of ddCherry and reduced overall levels of mitophagy in longterm cultures (up to DIV 42). These findings suggest that ISG expression can acutely disrupt protein turnover in neurons and that longterm ISG expression in neurons can impair mitophagy. We are currently determining whether these effects are ISG15-dependent and how this

process affects longterm neuron viability in vitro and in vivo.

Non-MS autoimmune diseases

#71 Effects of Initial BMI on Clinical Presentation and Prognosis in Neuromyelitis Optica Spectrum Disorder Patients

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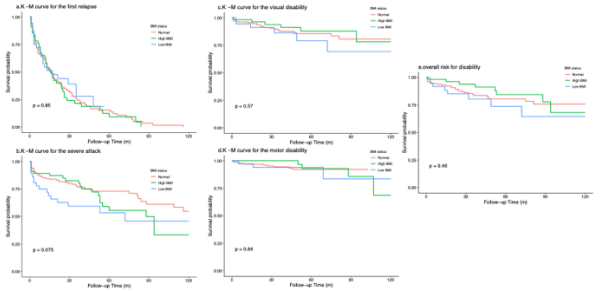
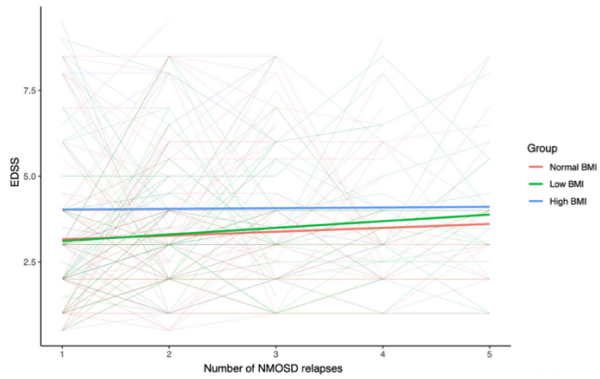
Objective: To investigate the correlation among body mass index (BMI) at onset, clinical features, and prognosis in patients with neuromyelitis optica spectrum disorder (NMOSD).

Method: This retrospective cohort studied patients with NMOSD from January 2015 to January 2022 and designated them into three groups based onBMI at NMOSD onset. The demographic and clinical records were reviewed and described. The Anderson-Gill proportional hazard model, Kaplan-Meier curves, and Cox proportional hazard models were used to evaluate the effect of BMI at onset on the risk of relapse and long-term outcomes.

Results: Of 246 patients with 799 NMOSD attacks included in this study, 36 patients had low BMI, 133 had normal BMI, 77 had high BMI, with a mean onset age of 40 ± 13 years, and the study population was 88% female. The medium follow-up time was 49 months; AQP4-IgG was found in 193 (78%) patients. Onset and relapse of area postrema syndrome (APS) were less frequent in patients with a normal BMI, and the annual relapse rate (ARR) after immunosuppressive therapy (IST) was significantly lower in patients with a low BMI. In the multivariable analysis, statistical correlation still existed between BMI at onset and risk of relapse (HR=1.03, 95% CI 1.03-1.03, p<0.001), risk of severe attack (HR=0.92, 95% CI 0.86-0.98, p=0.013), risk of visual disability (HR=0.9, 95% CI 0.81-1, p=0.047), and overall risk of disability (HR=0.89, 95% CI 0.82-0.98, p=0.015) after adjusting various variables.

Conclusion: Lower BMI at onset was associated with less frequent relapse but poor prognosis.





Keyword: *Neuromyelitis Optica spectrum disorders, Body mass index, Prognosis, Disability*

#72 Visual Disability in Neuromyelitis Optica Spectrum Disorders: Prognostic Prediction Models

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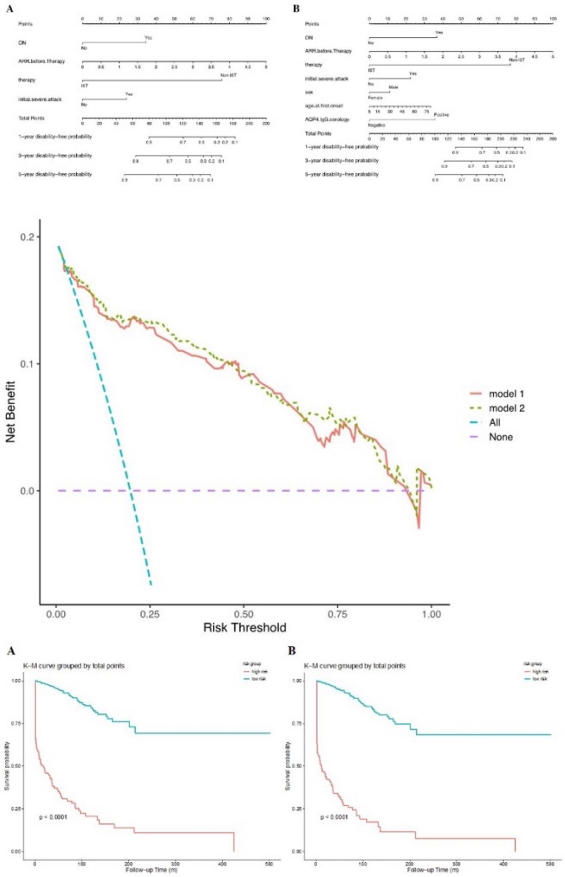
Background and Objectives: Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune inflammatory disease of the central nervous system characterized by simultaneous or consecutive episodes of acute optic neuritis and transverse myelitis. Attacks of NMOSD can result in the accrual of severe visual disability over time. This study aimed to develop and validate prognostic models for visual disability risk within 1, 3, and 5 years.

Methods: Medical records of NMOSD patients were retrospectively analyzed. The least absolute shrinkage and selection operator (LASSO) regression algorithm and univariate and multivariate Cox regression analyses were performed to select predictors of visual disability. Two models predicting the probability of visual disability in 1, 3, and 5 years were developed based on different selections and displayed as nomograms. Risk scores were calculated for every patient, and a cut-off point was obtained to recognize patients at high risk.

Results: In total, 161 (25.2%) patients developed visual disabilities during the follow-up period. Four visual disability-related factors were selected using LASSO regression: optic neuritis (ON) onset, higher annual relapse rate (ARR) before maintenance therapy, no maintenance immune suppression therapy (IST), and initial severe attack. Three additional predictors were determined using multivariate Cox regression: male sex, age at first onset, and positive AQP4-IgG serology. Discrimination and calibration were satisfied, with concordance indexes (C-index) close to 0.9 in both models. Decision curve analysis showed good clinical usefulness in both models, and Kaplan-Meier curves showed satisfactory discrimination between patients with high risk and low risk by the cut-off points.

Conclusion: This study reported predictors of visual disability and generated nomograms. High-risk patients need more active treatment and management to avoid unfavorable outcomes.



Keyword: *Neuromyelitis Optica spectrum disorders, Prediction models, Nomogram, Prognosis, Visual disability*

#73 Clinical Features and Prognosis of Tibetan Patients with Neuromyelitis Optica Spectrum Disorder are Different from those of Han Chinese Patients

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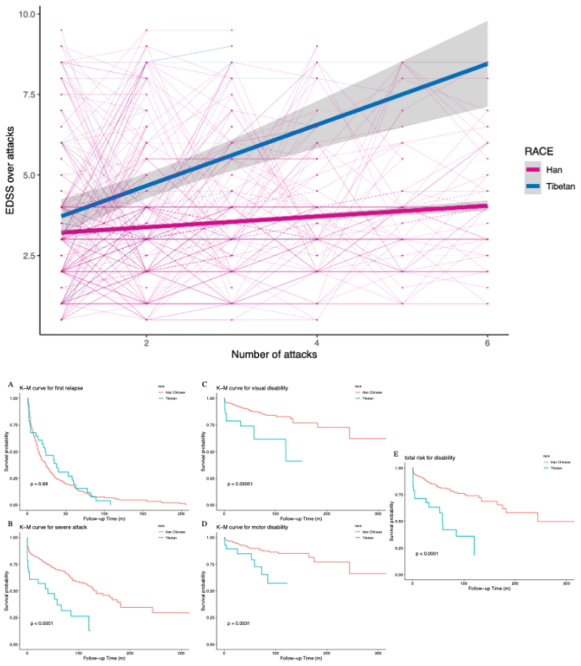
Background: To describe the prognosis of Tibetan patients with neuromyelitis optica spectrum

disorder (NMOSD) and compare it with that of Han Chinese patients.

Methods: The records of Tibetan patients with NMOSD were reviewed. Demographic and clinical data were described, and the Expanded Disability Status Scale (EDSS) score at each attack, response to immunosuppressive therapy, risk of first relapse, severe attack, visual disability, motor disability, and total risk of disability were compared with those of Han Chinese patients.

Results: A total of 376 patients with NMOSD were included in the study (28 Tibetans and 378 Han Chinese) with a mean onset age of 40±13 years and a median disease duration of 51 months; 89% of the patients were female. The most affected areas were the optic nerve (48%) and spinal cord (76%); none of the Tibetans developed area postrema syndromes. A total of 309 (82%) patients experienced at least one relapse, 145 (39%) experienced severe attacks, 56 (15%) and 46 (12%) developed permanent visual and motor disabilities, respectively. The total disability rate was 23% (n = 88). Tibetan patients showed higher EDSS during acute attacks compared to those of Han Chinese patients. Annual relapse rate did not differ between the two races. Risk of severe attack (HR=2.46, 95% CI:1.49-4.06), visual disability (HR=2.65, 95% CI:1.29-5.45), and total risk of disability (HR=2.81, 95% CI:1.59-4.98) were higher in Tibetan patients than in Han Chinese patients.

Conclusion: Tibetan patients with NMOSD have a higher risk of poor prognosis than Han Chinese patients.



Keyword: *Neuromyelitis Optica spectrum disorders, Tibetan, Race, Prognosis*

#136 Bone marrow granulopoiesis in neuromyelitis optica spectrum disorder

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B cells drive the immunopathology of neuromyelitis optica spectrum disorder (NMOSD), an autoimmune neurological disease with autoantibodies to anti–aquaporin-4 (AQP4-IgG) in 75% of patients. Bone marrow hematopoietic system can sense immune activation and generate hematogenous cells to orchestrate inflammation. Yet the alterations of bone marrow hematopoietic cells in NMOSD and their potential impact on disease progression remain unknown. We show remarkably augmented granulopoiesis in bone marrow of NMOSD patients, accompanied by expansion of B cell clones. This aberrant granulopoiesis was

mediated by JAK-STAT pathway and led to dramatic production of BAFF that drives the production of antibody-secreting cells and AQP4-IgG, which is also observed in NMOSD patients with relapse after receiving rituximab. In an open-label, single-arm trial of belimumab, a monoclonal antibody against BAFF, in 14 NMOSD patients, belimumab reduced relapse rate, antibody-secreting cells and AQP4-IgG. Thus, targeting bone marrow niche may present a new avenue to treat NMOSD.

Keyword: *Bone marrow, Hematopoietic stem cell, Granulopoiesis, B cell, neuromyelitis optica*

#206 Efficacy and Safety of Ravulizumab in Adults With Anti-aquaporin-4 Antibody-positive Neuromyelitis Optica Spectrum Disorder: Outcomes From the Phase 3 CHAMPION-NMOSD Trial

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Background and aims: Ravulizumab binds the same complement component 5 epitope as eculizumab with a longer half-life, enabling an extended dosing interval (8 versus 2 weeks). We report efficacy/safety outcomes from the CHAMPION-NMOSD (NCT04201262) phase 3 study assessing ravulizumab in anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder (AQP4+ NMOSD).

Methods: Adults received a ravulizumab weight-based intravenous loading dose followed by maintenance dosing on day 15 and every 8 weeks thereafter. Because eculizumab availability precluded the ethical use of concurrent placebo control, the placebo arm of PREVENT (NCT01892345; eculizumab in NMOSD) served as an external comparator. The primary endpoint was time to first adjudicated on-trial relapse and relapse risk reduction (RRR). Secondary endpoints were adjudicated on-trial annualized relapse rate (ARR) and Hauser Ambulation Index (HAI) worsening.

Results: Median (range) follow-up was 73.5 (11.0–117.7; n=58) weeks with ravulizumab and 36.0 (1.9–117.7; n=47) weeks with placebo. The primary endpoint was met; no adjudicated relapses occurred with ravulizumab versus 20 with placebo (RRR: 98.6%; P<0.0001). ARR (upper 95%CI) was 0.00 (0.04) with ravulizumab, which was superior to predefined comparator ARR (0.25; P<0.0001). Fewer patients experienced clinically important HAI worsening with

ravulizumab (2/58 [3.4%]) than with placebo (11/47 [23.4%]; P=0.023; OR: 0.16 [95%CI: 0.03–0.77]). TEAEs were reported in 93.1% (ravulizumab) and serious AEs in 13.8% of patients; 2 vaccinated patients experienced meningococcal infection (2.4/100 patient-years) and recovered, and 1 continued in the trial. No deaths occurred. Despite longer follow-up (median treatment: 90.9 weeks), efficacy and safety remained consistent with that in the primary treatment period, with no relapses (RRR: 98.7%).

Conclusions: Ravulizumab significantly lowered relapse risk and HAI worsening versus placebo in AQP4+ NMOSD, an effect unaltered by differences in baseline characteristics. No changes to the known ravulizumab safety profile were identified.

#211 A protective effect of lower MHC-II expression in MOGAD

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Introduction:

Myelin oligodendrocyte glycoprotein-antibody-associated disease (MOGAD) is an autoimmune inflammatory disorder of the central nervous system (CNS). MOGAD patients can have a monophasic or relapsing disease course, with a relapsing course reported in approximately half of patients with MOGAD. Our study aimed to identify which immunological pathways are altered in MOGAD and whether they can predict disease progression.

Design and Methods:

Expression levels of immunological genes were analyzed in peripheral blood mononuclear cells (PBMCs) of MOGAD patients, multiple sclerosis (MS) patients, neuromyelitis optica spectrum disorder (NMOSD) patients, and healthy controls (HCs) (MOGAD: n = 41; MS: n= 23; NMOSD: n= 23; and HC: n=56) using the nanostring nCounter technology and validated using reverse transcription polymerase chain reaction (RT-PCR). Volumetric brain information was determined in brain magnetic resonance imaging (MRI) using volBrain and MDbrain platforms.

Results

We identified 35 genes that differentiated between MOGAD patients and HCs. Specifically, the expression of genes related to the major histocompatibility complex (MHC)-II pathway was reduced and the expression of STAT3 and IL-10 that regulate MHC-II expression was increased. Several cytokines and cytokine receptors were upregulated in the MOGAD group, including IL-8, IL23, and CSF1.
We found a significant negative correlation between HLA-DRA expression and brain volume of MOGAD patients and noted that HLA-DRA expression levels were downregulated in monophasic compared to relapsing MOGAD patients. Also, PBMCs and oligodendrocyte progenitor cells (OPCs) cultured with serum from MOGAD patients exhibited decreased MHC-II expression compared with serum from HCs.

Discussion: We found reduced expression of genes related to MHC-II in MOGAD patients. The MHC-II expression levels correlate with disease activity factors, including time to second relapse and monophasic disease. Understanding the underlying mechanism related to MHC II

presentation in MOGAD patients may lead to the development of new therapeutic approaches.

Keyword: *MOGAD, MHC-II, Relapsing MOGAD, Nanostring*

#213 IL-6 as a blood biomarker for NMOSD disease activity

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 # These authors contributed equally to the manuscript and share last authorship

Background: Neuromyelitis Optica spectrum disorder is a rare demyelinating disorder that preferentially affects the spinal cord and optic nerve. There are no valid clinical or laboratory methods to predict disease progression, disability outcome, and relapse rate. Interleukin 6 (IL-6) is a proinflammatory cytokine elevated in the serum and CSF of NMOSD patients. Our study aimed to determine if serum levels of IL-6 could serve as a biomarker for NMOSD disease activity.

Methods: We evaluated the serum levels of IL-6 in 26 NMOSD patients at various disease pivot points, using enzyme-linked immunosorbent assay. We correlated serum IL-6 levels to brain MRI volumetric measures using volBrain software and to Expanded Disability Status Scale, NMO-preventing treatments, and clinical subtypes (relapse and remission states).

Results: NMOSD patients at relapse had higher levels of IL-6 than those in remission. No differences in the serum levels of IL-6 were found between NMOSD patients in remission and HCs. IL-6 levels at relapse positively correlate with CSF total protein levels during relapses. Furthermore, IL-6 Levels at relapse negatively correlate with the volume of total grey matter, whole Brain, cerebellum, brain stem, thalamus, and putamen.

Conclusion: Our finding suggests that serum levels of IL-6 might serve as a biomarker for disease activity (e.g., relapse vs. remission). The association between increased levels of IL-6 and reduced brain volume suggests that the IL-6 signaling pathway may mediate disability.

Keyword: *NMOSD, IL-6, Brain MRI, NMOSD relapse*

#216 Study of autoreactive CD4+ T cells in recent-onset Narcolepsy

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Narcolepsy type 1 (NT1) is a rare, chronic and disabling neurological disease, causing excessive daytime sleepiness and cataplexy. NT1 is characterized pathologically by an almost complete loss of neurons producing the hypocretin (HCRT)/orexin neuropeptides in the lateral hypothalamus. Genetic and environmental factors strongly suggest the involvement of the immune system in the loss of orexin neurons. Studies showing the presence of autoreactive $CD4^+$and $CD8^+$ T cells in the blood of NT1 patients support this hypothesis. Giving the uniquely strong genetic association of the HLA-DQB1*06:02 allele with NT1 (RR > 200), we

hypothesized that CD4+ T cells play a central role in NT1 pathogenesis.
We, therefore, used an experimental approach consisting of in vitro expansion of antigen-specific CD4+ memory T cells from the blood of persons with NT1 or controls. Two approaches to screen for autoreactive T cells: (i) A confirmatory approach using peptides from HCRT (HCRT54-66 and HCRT-NH54-66) that have previously been shown to elicit autoreactive T cell responses; (ii) A discovery approach using the whole pre-pro-HCRT precursor protein. After 14 days of culture of sorted memory CD4+ T cells with the antigens, the frequency of antigen-specific T cells was analyzed based on their proliferation (CTV dilution) and their function (measuring multiple cytokines in supernatant of cultures with a cytokine multiplexing assay). Screening of 23 NT1 patients close to disease onset (all HLA-DQB1*06:02+) and age- and sex-matched 16 healthy donors carrying the HLA-DQB1*06:02 allele revealed similar frequencies of autoreactive CD4+ T cells specific to HCRT-NH54-66 and pre-pro-HCRT in NT1 patients and healthy donors. However, higher frequencies of autoreactive T cells specific to HCRT54-66 were found in NT1 patients compared to healthy donors. Furthermore, high levels of GM-CSF were detected in the supernatant of HCRT54-66-stimulated CD4+ T cells from NT1 patients, but not in unstimulated supernatant. To gain insight into the potential pathogenic mechanisms that could drive autoreactive T cells to trigger the loss of orexin, we sorted CD4+ memory T cells specific to HCRT54-66 and performed transcriptomic analysis. As an additional approach to assess the potential pathogenic effect of GM-CSF in NT1, we took advantage of our immune-mediated Narcolepsy mouse model to neutralize this cytokine and determine the effect on the orexinergic neuronal loss.
Collectively, our data aim at refining our understanding of the autoimmune pathways involved in NT1 pathogenesis.

Keyword: *Narcolepsy, T cell, Autoreactive, GM-CSF*

#217 Cerebrospinal fluid proteomics in recent-onset Narcolepsy type 1 reveals activation of the complement system

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Narcolepsy type 1 (NT1) is a rare, chronic and disabling neurological disease causing excessive daytime sleepiness and cataplexy. NT1 is characterized pathologically by an almost complete loss of neurons producing the hypocretin/orexin neuropeptides in the lateral hypothalamus. Genetic and environmental factors strongly suggest the involvement of the immune system in the loss of orexinergic neurons. The cerebrospinal fluid (CSF), secreted locally and surrounding the central nervous system (CNS), represents an accessible window into CNS pathological processes.

To gain insight into the biological and molecular changes in NT1 patients, we performed a comparative proteomics analysis of the CSF from 21 recent-onset NT1 patients and from two control groups: group 1 with somatoform disorders, and group 2 patients with hypersomnia other than NT1, to control for any potential effect of sleep disturbances on CSF composition. To achieve an optimal proteomic coverage analysis, the twelve most abundant CSF proteins were depleted, and samples were analyzed by nano-flow liquid chromatography tandem mass spectrometry (nano-LC-MS/MS) using the latest generation of hybrid Orbitrap mass spectrometer. Our study allowed the identification and quantification of up to 1943 proteins, providing a remarkably deep analysis of the CSF proteome. Interestingly, gene set enrichment analysis indicated that the complement and coagulation systems were enriched and significantly activated in NT1 patients in both cohorts analyzed. Notably, the lectin and alternative complement pathway as well as the downstream lytic membrane attack complex were congruently increased in NT1.

Our data suggest that the complement dysregulation in NT1 patients can contribute to immunopathology either by directly promoting tissue damage or as part of local inflammatory responses. We, therefore, reveal an altered composition of the CSF proteome in NT1 patients, which points to an ongoing inflammatory process contributed, at least in part, by the complement system.

Keyword: *Narcolepsy, Proteomics, Cerebrospinal fluid, Complement*

#253 Comparison of different apheresis methods in patients with immune mediated neurological diseases

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Immune-mediated neurological diseases, such as Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), Guillain-Barre syndrome (GBS) and Multiple Sclerosis (MS) are characterized by axonal or myelin damage due to autoimmunological processes. Therapeutic apheresis is a commonly used therapy, that removes pathogenic agents such as autoantibodies, inflammatory cytokines or complement from the patient's blood. Although clinically relevant, it is not yet known whether different methods of apheresis, namely plasma exchange (PE) and immunoadsorption (IA), do have a specific impact on protective and destructive serum factors as well as on clinical outcome parameters.

Patients with immune-mediated neurological conditions (CIDP, GBS, MS; n=75) and controls (n=16) before, during and after apheresis therapy were included. The cohort consists of 43 women and 32 men with a median age of 50 (20 to 89). Pro- and anti-inflammatory cytokines, neurotrophic factors, hormones, and vitamins were measured in patient's blood samples. Immune cell subpopulations were determined using flow cytometry.

The preliminary results show a correlation between the therapeutic procedures and a wide variety of pro/anti-inflammatory and destructive/protective factors. In particular, the first apheresis effectively eliminates immunogenic plasma factors, with PE being more effective than IA. Blood pressure drops occur

with both procedures but are more severe with PE. Hemoglobin and fibrinogen also decrease more with PE than with IA. However, IA preserves more vitamins and may even increase serum levels of hepatocyte growth factor (HGF) and IL-10 – considered to be neuroprotective and anti-inflammatory - compared to PE.

PE eliminates plasma proteins but also neuroprotective factors more strongly than IA. Overall, patients with IA had fewer side effects. A better understanding of the effects caused by different apheresis methods may help to improve patient's outcome regarding a specialized therapy dependent on patients' characteristics. Some may benefit from a more specific removal of destructive factors while others may rely on the preservation of protective and regenerative factors in an acute therapy for inflammatory conditions in neurology.

Keyword: *Apheresis, CIDP, GBS, MS*

#293 Pharmacokinetics, pharmacodynamics, and safety of efgartigimod in healthy Chinese adults

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Background

The intravenous (IV) formulation of efgartigimod, a human immunoglobulin G (IgG) 1–derived Fc fragment targeting the neonatal Fc receptor, is approved in the USA, the EU, and Japan for generalized myasthenia gravis treatment. A subcutaneous (SC) formulation co-formulated with recombinant human hyaluronidase PH20 is under development as an alternative. We report the pharmacokinetic (PK), pharmacodynamic (PD), and safety of IV and SC efgartigimod (Efgar IV and Efgar PH20 SC) in healthy Chinese adults.

Methods

Healthy Chinese adults (age 18-65 years) were eligible to participate in two independent, randomized, double-blind, placebo-controlled phase 1 studies (CTR20211952/CTR20211805). Subjects were randomized 3:1 to receive Efgar IV 10 mg/kg, Efgar PH20 SC 1000 mg, or matching placebo once every 7 days for 4 doses. The primary comparison was based on PK parameters.

Results

In each IV and PH20 SC study, 16 subjects were enrolled and included in the PK analysis. After the first IV infusion, a mean C_{max} of 183 µg/mL was reached; mean $AUC_{0\text{-}168h}$ was 4620 µg·h/mL. After the first SC injection, a mean C_{max} of 27.2 µg/mL was achieved with a median (range) T_{max} of 47.9 h (23.7-72.0); mean $AUC_{0\text{-}168h}$ was 3300 µg·h/mL. Accumulation ratio based on AUC was 1.15 and 1.52 with IV and SC administration, respectively. Maximal mean (standard deviation) reductions from baseline in total IgG levels of 60.7% (2.0) for Efgar IV and 66.4% (1.4) for Efgar PH20 SC were reached approximately 24 days after the first dose.

There were 4 (33.3%) subjects in the IV group and 6 (50.0%) subjects in the PH20 SC group that developed treatment-induced anti-drug antibodies (ADAs) against efgartigimod. No apparent impact of ADAs on PK, PD, or safety was observed. Treatment-emergent adverse events (TEAEs) were reported in 2 (50.0%), 9 (75.0%), and 3 (75.0%) subjects in the IV placebo, Efgar PH20 SC, and SC placebo groups, respectively. Treatment-related AEs (TRAEs) were reported in

7 (58.3%) subjects receiving Efgar PH20 SC; those occurring in >1 subject were related to injection-site reactions. There was 1 TRAE (increased bilirubin) in 1 (25.0%) subject in the SC placebo group. An adverse event of special interest (AESI), oral herpes, was reported in 1 (8.3%) subject in the Efgar PH20 SC group, and upper respiratory tract infection was reported in 1 (25.0%) subject (SC placebo group). No TRAEs or AESIs were reported in the IV study. No albumin reduction, lipid elevation, or edema were seen in either study. All TEAEs were mild or moderate and resolved.

Conclusions

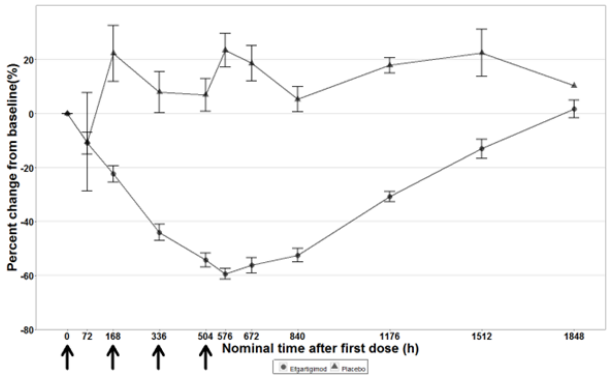
The PK, PD, and safety profile of efgartigimod in healthy Chinese adults was similar to the known profile of efgartigimod in non-Chinese subjects. Both the IV and SC formulations effectively reduced total IgG levels in a similar percentage. Both formulations of efgartigimod were safe and well tolerated in healthy Chinese adults.

Table 1. Pharmacokinetic parameters after efgartigimod IV 10 mg/kg infusions and efgartigimod PH20 SC 1000 mg injections

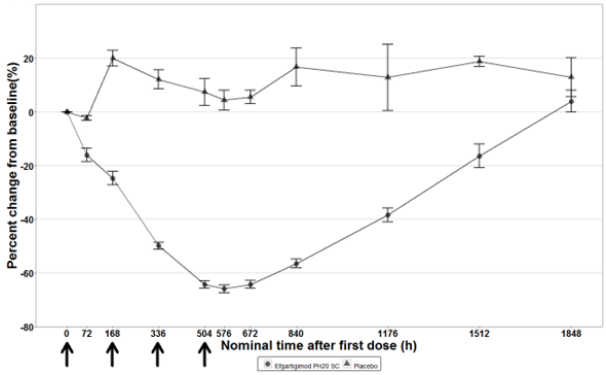
	After the first dose of efgartigimod IV 10 mg/kg (N=12)	After the first dose of efgartigimod PH20 SC 1000 mg (N=12)
T_{max} (h)	1.0 (1.0, 1.0)	47.9 (23.7, 72.0)
C_{max} (µg/mL)	183 (19.6)	27.2 (8.8)
$AUC_{0\text{-}168h}$ (µg·h/mL)	4620 (335)	3300 (978)
	After the fourth dose of efgartigimod IV	After the fourth dose of efgartigimod PH20 SC
	10 mg/kg (N=12)	1000 mg (N=12)
T_{max} (h)	1.0 (1.0, 1.0)	47.7 (11.7, 47.8)
C_{max} (µg/mL)	194 (19.4)	42.1 (13.2)
C_{trough} (µg/mL)	8.6 (2.0)	14.3 (3.9)
AUC_{τ} (µg·h/mL)	5300 (617)	4790 (906)
$t_{½}$(h)	92.7 (6.4)	84.3 (12.7)
CL (L/h)	0.122 (0.0186)	NA
CL/F (L/h)	NA	0.216 (0.0387)
V_{ss} (L)	10.4 (1.6)	NA
Vz/F (L)	NA	25.9 (5.1)
R_{AC_Cmax}	1.07 (0.111)	1.60 (0.337)
R_{AC_AUC}	1.15 (0.114)	1.52 (0.276)

$AUC_{0\text{-}168h}$=area under the concentration-time curve from time 0 to time 168 h post dose; AUCτ=area under the concentration-time curve during the dosing interval of 168 h; CL=clearance; CL/F=apparent clearance; C_{max}=maximum observed concentration; C_{trough}=concentration at the end of dosing interval; IV=intravenous; N=number of subjects for the analyses of PK parameters; NA=not applicable; PK=pharmacokinetic(s); R_{AC}=accumulation ratio; R_{AC_AUC}=R_{AC} based on AUC_{τ}; R_{AC_Cmax}=R_{AC} based on C_{max}; SC=subcutaneous; SD, standard deviation; $t_{½}$=terminal half-life; T_{max}=time to reach C_{max}; Vss=volume of distribution at steady state; Vz/F=apparent volume of distribution.
Note: Median (minimum, maximum) for T_{max} and mean (SD) for other parameters.
The IV infusion time was 1 hour.

Figure 1. Percentage change from baseline in serum total IgG level after 4 administrations of efgartigimod (a) IV 10 mg/kg and (b) PH20 SC 1000 mg (pharmacodynamic analysis set)
(a)



(b)



h=hour; IgG=immunoglobulin G; IV=intravenous; SC=subcutaneous.
Each arrow indicated the timepoint of each study drug administration.

Keyword: *efgartigimod, pharmacokinetics, pharmacodynamics, safety, healthy Chinese adults*

#294 Mechanisms of neurodegeneration in a human in vitro disease model of anti-IgLON5 autoimmune encephalitis

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Anti-IgLON5 autoimmune encephalitis is a chronic neurological disorder. Pathological hallmarks are autoantibodies against the cell adhesion molecule IgLON5, neurodegeneration and accumulation of hyperphosphorylated tau. Patients manifest heterogeneous symptoms including sleep disorders, gait abnormalities and cognitive dysfunction. We investigated the effects of anti-IgLON5 antibodies on human neurons. Human neural precursor cells were differentiated for 21 days and exposed to isolated IgG fraction of either patients with confirmed anti-IgLON5 antibodies or healthy age matched controls for 7 days. Unbiased proteome analysis revealed differentially expressed proteins in anti-IgLON5 IgG treated cells compared to controls. Live cell imaging was performed to investigate cell death and calcium homeostasis. Interestingly, proteomic data showed different clusters of significantly regulated proteins among the anti-IgLON5 IgG treated cultures, highlighting the heterogeneity of the disease. Differentially expressed proteins of anti-IgLON5 IgG treated neurons compared to controls especially clustered around gene ontology terms like cytoskeleton, mitochondria and endoplasmic reticulum. Furthermore, we could detect increased cell death and disrupted calcium homeostasis in anti-IgLON5 IgG treated neurons. In conclusion, anti-IgLON5 antibodies lead to neurodegeneration and cell death in human neurons in vitro, supporting the hypothesis that in addition to autoimmunity there is a neurodegenerative component emanating from anti-IgLON5 antibodies.

Keyword: *IgLON5, autoimmune encephalitis, neurodegeneration*

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#327 Identification of clonally expanded CD8+ T cells in autoimmune encephalitis after co-culture with autologous neurons and astrocytes

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Introduction

Antibodies targeting brain surface, synaptic or intracellular antigens are an essential hallmark for the diagnosis of autoimmune encephalitis (AIE). Unlike CNS cell surface antibodies, intracellular brain-targeting antibodies are probably not key effectors of disease as they are unable to reach their antigen. Importantly, AIE linked with intracellular antigens (I-AIE) (e.g. Hu, Ri or GFAP AIE) appear with a concomitant cytotoxic CD8+ T cell response which is thought to be responsible for disease development. Our objective here, is to screen patients with I-AIE for the presence of brain-reactive CD8+ T cells.

Method

To expand and detect brain-reactive CD8+ T cells, we have developed a co-culture assay between peripheral blood mononuclear cells (PBMCs) and autologous human-induced pluripotent stem cell (hiPSC)-derived neurons and astrocytes. First, an expansion step is initiated where ex vivo PBMCs and autologous HLA-I-enhanced neurons or astrocytes are cultured together for 14 days. Second, expanded CD8+ T cells are isolated and incubated overnight with fresh HLA-I-enhanced neurons and astrocytes and reactive CD8+ T cells are detected by secretion of IFN-gamma. Finally, if an IFN-gamma production is present, CD8+ T cells undergo bulk TCR sequencing to assess for clonally expanded TCR-alpha and TCR-beta chains.

Results

First, we have generated hiPSC-derived neurons and astrocytes from 6 healthy donors (HD) and 3 patients with I-AIE (1 Hu AIE, 1 Ri AIE and 1 GFAP AIE). Second, we demonstrate that hiPSC-derived neurons and astrocytes upregulate HLA class I expression upon exposure to IFN-gamma and TNF-alpha. Third, we are able to detect neuron or astrocyte-reactive CD8+ T cells in our cohort after co-culture with autologous target cells. Finally, in I-AIE patients we identify TCR-alpha and TCR-beta chains that are present at a high frequency ex vivo and then clonally expanded after co-culture with autologous neurons or astrocytes.

Discussion

Overall, our co-culture system allows to successfully identify neuron and astrocyte-reactive CD8+ T cells from virtually any donor. We are currently performing TCR-specificity validation assays from identified clonally expanded TCR-alpha and TCR-beta chain sequences of I-AIE patients. The identification of pathogenic TCRs implicated in I-AIE is essential for the identification of antigens implicated in disease development and will undoubtedly deepen our understanding of this poorly treatment-responsive disease.

Keyword: *CD8+ T cells, autoimmune encephalitis, TCR, neurons, astrocytes*

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#380 Selective myofiber vulnerability and immune cell diversity in inclusion body myositis

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Inclusion body myositis (IBM) is a treatment-refractory, slowly progressing idiopathic inflammatory myopathy (IIM) and the most common IIM among the elderly. IBM is characterized by a pronounced cytotoxic T cell-driven inflammation and concurrent myofiber degeneration and atrophy. To this day the pathogenesis of IBM remains poorly understood limiting the development of therapies which could halt the progression of the disease. Here, we collected snap-frozen muscle biopsies of the quadriceps femoris muscle of healthy control patients, patients with immune-mediated necrotizing myopathy (IMNM), and patients with IBM and utilized single-nucleus RNA sequencing and spatial transcriptomics to identify disease-specific transcriptomic alterations on a cellular level and trace them back to the muscle section. Moreover, this enabled us to explore the diversity of innate and adaptive immune cell types and observe homeostatic and reactive myonuclei subtypes in IBM. More specifically, we were able to discover a disease-specific subtype of myonuclei which showed to be spatially correlated with T cell-driven inflammation. Further, validation by multiplex in situ hybridization revealed, that these myonuclei were mostly present in type IIA myofibers suggesting a vulnerability of type IIA myofibers to specific cell stress pathways in IBM. This finding offers new insights into the pathogenesis of IBM and might break ground towards new biomarkers and cell type-specific targeted therapeutic interventions in IBM.

Psychoneuroimmunology

#84 Implication of angiotensin II signaling through endothelial AT1aR in susceptibility to psychological trauma in a mouse model of PTSD

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Background: Posttraumatic stress disorder (PTSD) affects over 9% of the population. The only FDA-approved drugs for treatment of PTSD have limited efficacy. It is therefore urgent to better understand the pathophysiological mechanisms of PTSD to developing new therapeutic avenues. Studies support a role of angiotensin II (Ang II) in the response to psychological trauma, leading to higher risk for PTSD. Still, the mechanisms underlying this relationship remain poorly understood. We hypothesized that following psychological trauma, Ang II binding to endothelial angiotensin type 1a receptors ($AT_{1a}Rs$) leads to BBB disruption and infiltration of inflammatory molecules into the brain, contributing to the onset of PTSD symptoms. **Methods**: Male and female wild-type (WT) mice and mice deficient for $AT_{1a}R$ in endothelial cells ($eAT_{1a}R^{-/-}$) were exposed to predator stress. A week later, anxiety-like and avoidance behaviors were evaluated using the open field (OF), light-dark box (LD), and trauma-reminder (TR) tests. Blood pressure (BP) was monitored by plethysmography at baseline, as well as 1 and 10 days after stress. **Results**: Across all three tests, predator stress increased anxiety-like and avoidance behaviors independently of $eAT_{1a}R$ phenotype in female mice ($p < 0.05$ or $p < 0.001$). In the LD test, $eAT_{1a}R^{-/-}$ phenotype reduced overall anxiety-like behavior in females regardless of stress exposure ($p < 0.05$). In males, in the TR test, stress exposure increased avoidance behavior ($p < 0.0001$). Only in males, the $eAT_{1a}R^{-/-}$ phenotype reduced anxiety-like and avoidance behavior in the LD ($p < 0.05$) and TR tests ($p < 0.05$). Surprisingly, $eAT_{1a}R^{-/-}$ mice had higher systolic and diastolic BP vs WT mice. This effect was observed in both sexes, with a stronger effect in males. **Conclusion:** These findings suggest that peripheral Ang II signaling through endothelial $AT_{1a}Rs$ is implicated in the development of overall anxiety and avoidance of trauma-reminder cues. Brain levels of inflammatory markers and BBB integrity will also be presented to provide additional key insights on the mechanisms underlying the association between Ang II and PTSD risk.

#383 Characterization of neuroinflammation in socially isolated female mice

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Depression is a psychiatric disorder that negatively impacts the quality of life of patients and in extreme cases can lead to suicide. About 4.4% of the world population suffers from this condition, which is also known to be more prevalent in the female population. The main hypothesis that explains depression etiology from a neurobiological point of view are the depletion of brain serotonin, dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis and alteration of the continuous production of adult-generated neurons in the dentate gyrus of the hippocampus. Recent evidence suggests that alteration in peripheral immune responses and neuroinflammation mediated by microglia are also key factors involved in depression pathogenesis, interacting with all three theories correlated with depression. Animal models chronically exposed to stressful stimuli can induce anxiety- and depressive- like behavior, becoming useful tools for studying the pathophysiology of depression, and for identifying new therapeutic targets. However, the type of stress, temporality, and gender differences are responsible for heterogenous data found in the literature, making necessary to understand neurobiological and immunological responses dysregulations in the context of each model. On the other hand, even though depression is more prevalent in the female population, studies using female models on the effects of stress on behavior and other biological outcomes are scarce. In this study, we investigated the effects of social isolation stress on adult female mice on, depressive and anxiety-like behavior through open field test (OFT), forced swim test (FST) and splash test (ST). In addition, we evaluated the inflammatory response by analyzing changes in peripheral T cell populations by flow cytometry. Neuroinflammation in the hippocampus was analyzed by detecting the expression of pro and anti-inflammatory cytokines using RT-qPCR, as well as astrocytic marker glial fibrillary acidic protein (GFAP) and microglial marker ionized calcium-binding adapter molecule 1 (IBA1) by western blot and immunohistochemistry. Our results indicate that after 8 weeks of social isolation, female mice show a robust depressive-like behavior, but not an anxiety-like behavior. We did not detect peripheral signs of inflammation, but we did observe neuroinflammation, as an increase in IL-1beta was detected in the hippocampus, in addition to an increase in the number of Iba1 positive cells, which exhibited thicker branches in the isolated females. The work presented contributes to our understanding of the impact of chronic stress and social isolation on neuroimmunity, and its association with depressive-like behavior in female mice.

Keyword: *Social Isolation, Depression, Female, Microglia*

August 22

Biomarkers of neuroinflammation

#57 Single-Cell Immune Profiling for Prediction of Multiple Sclerosis Severity over Five Years

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Background: The disease trajectory of multiple sclerosis (MS) is unpredictable. The identification of novel readily measurable biomarkers is crucial for improved patient care. Combining single-cell RNA-sequencing (scRNA-Seq) and high-parameter flow cytometry analysis of well-characterized biobanked samples can facilitate the discovery of biomarkers.

Objective: To identify novel protein biomarkers using peripheral blood mononuclear cells (PBMCs) to predict MS disease outcome.

Methods: A 21-color flow cytometry panel was designed based on differentially expressed genes identified from scRNA-Seq data of three untreated MS patients and three healthy controls (HCs). This panel was applied to biobanked PBMC samples from 35 HCs and 52 treated and untreated MS patients for a longitudinal retrospective study design, in which the flow cytometry data was correlated with the five-year clinical outcome post-sampling. Unsupervised FlowSOM clustering was performed to delineate cell populations. The proportion of FlowSOM clusters and the normalized median fluorescence intensity, representing protein expression, of seven markers of interest (S100A8/A9, lysozyme, beta2-microglobulin, CD69, IL7 receptor [IL7R], CD18, DAP12) within each cluster were correlated with clinical outcome measures. Three-parameter no evidence of disease activity (NEDA-3) was achieved by MS patients if they had no relapse, no new lesion, or no progressing disability score over the course of the study.

Results: MS patients could be discriminated from HCs by altered proportions of subpopulations of T cells (log2 fold change [FC] = -1.01, p<0.001), classical (CD14hiCD16-) monocytes (log2FC = 0.98, p<0.0001), and intermediate (CD14+CD16+) monocytes (log2FC = 2.57, p<0.001). In addition, CD69 was widely overexpressed in MS patients across most cell populations, suggesting a global increase in immune activation. Among MS patients, those who did not achieve NEDA-3 had increased levels of S100A8/A9 and IL7R in intermediate monocytes and classical monocytes. Specifically, the increased expression of the heterodimer S100A8/A9 in classical, intermediate, and non-classical (CD16hiCD14lo) monocytes was predictive of radiological activity (one or more new lesions over five years, all p<0.05). Furthermore, the expression of S100A8/A9 and IL7R tended to correlate with the severity of disease activity.

Conclusion: High-dimensional single-cell immune profiling could predict MS disease severity over five years.

Keyword: *Multiple Sclerosis, Flow Cytometry, Biomarkers*

#92 Magnetic transferrin nanoparticles (MTNs) assay as a novel isolation approach for exosomal biomarkers in neurological diseases

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Background : Brain-derived exosomes released into the blood are considered a liquid biopsy to investigate the pathophysiological state, reflecting the aberrant heterogeneous pathways of pathological progression of the brain in neurological diseases. Brain-derived blood exosomes provide promising prospects for the diagnosis of neurological diseases, with exciting possibilities for the early and sensitive diagnosis of such diseases. However, the capability of traditional exosome isolation assays to specifically isolate blood exosomes and to characterize the brain-derived blood exosomal proteins by high-throughput proteomics for clinical specimens from patients with neurological diseases cannot be assured. We report a magnetic transferrin nanoparticles (MTNs) assay, which combined transferrin and magnetic nanoparticles to isolate brain-derived blood exosomes from clinical samples.

Methods : The principle of the MTNs assay is a ligand-receptor interaction through transferrin on MTNs and transferrin receptor on exosomes, and electrostatic interaction via positively charged MTNs and negatively charged exosomes to isolate brain-derived blood exosomes. In addition, the MTNs assay is simple and rapid (< 35 min) and does not require any large instrument. We confirmed that the MTNs assay accurately and efficiently isolated exosomes from serum samples of humans with neurodegenerative diseases, such as dementia, Parkinson's disease (PD), and multiple sclerosis (MS). Moreover, we isolated exosomes from serum samples of 30 patients with three distinct neurodegenerative diseases and performed unbiased proteomic analysis to explore the pilot value of brain-derived blood protein profiles as biomarkers.

Results : Using comparative statistical analysis, we found 21 candidate protein biomarkers that were significantly different among three groups of neurodegenerative diseases.

Conclusion : The MTNs assay is a convenient approach for the specific and affordable isolation of extracellular vesicles from body fluids for minimally-invasive diagnosis of neurological diseases.

#173 Alterations of brain extracellular matrix during experimental autoimmune encephalomyelitis

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In multiple sclerosis (MS) and in its animal model, experimental autoimmune encephalomyelitis (EAE), both remodeling of the brain extracellular matrix (ECM) as well as softening of the brain following inflammation has been reported (1,2). Alterations in chondroitin sulfate (CS) proteoglycans are shown to impair remyelination in MS and EAE (3). However, how

neuroinflammation affects CS glycosaminoglycans (GAGs) and the influence of ECM remodeling on brain stiffness remains unknown.

Profiling of CS/HA using 2-aminobenzamide-HPLC and gene expression analysis of GAG-relevant pathways were cross-sectionally carried out in the brains of female SJL mice with EAE immunized with PLP139-151 and adjuvants (n=6 per timepoint). Sham-immunized mice were used as controls. To assess brain stiffness, in vivo multifrequency MRE was conducted longitudinally (n=15) and cross-sectionally (n=8) in a 7T preclinical MRI. The resulting parameter maps based on stiffness were registered to a reference atlas and segmented. Histological assessment of tissue inflammation and WFA-labeled perineuronal nets (PNNs) was conducted cross-sectionally in different EAE phases. Furthermore, a pilot study in MS patients (n=12) and healthy controls (n=14) assessed regional softening as clinical imaging marker.

Significant changes were observed in the cerebellum of EAE mice for genes involved in GAG metabolism, starting at pre-onset with downregulation of Bcan, Cspg5, and upregulation of Has2, Gusb, Hexb, Hpse. GAG quantification at peak EAE revealed an altered sulfation profile, with CS/DS-4S decreased by 3,6% and HA-0S increased by 26,1% at peak, both reducing in the remission phase. MRE revealed a pronounced softening of the cortex which correlated with disease course and was associated with an increase in $Iba1^{+}$ cells. Loss of cortical CS in the perineuronal nets (PNN) was detected during EAE and correlated with reduction of tissue stiffness and disease score. Similarly, cortical areas are primarily affected by MS-related softening in patients. As PNNs integrity has been shown to contribute to structural and functional changes in both in EAE and MS, cortical MRE emerges as a marker of active neuroinflammation to follow disease progression.

Remodeling of the brain ECM, as seen by the altered composition of CS/HA GAGs and loss of PNN integrity describe the inflammatory phases of EAE, affecting the mechanical properties of the brain. These changes may have an impact on disease progression and can be targeted for imaging of ongoing inflammation in MS using MRE.

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Keyword: *experimental autoimmune encephalomyelitis, glycosaminoglycans, chondroitin sulfate, perineuronal nets, extracellular matrix*

#223 Comparative Analysis of microRNA Distribution in MS, NMOSD, and Healthy Controls: Implications for Diagnostic Accuracy

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Introduction

CNS demyelinating diseases encompass conditions such as Multiple Sclerosis (MS), and Neuromyelitis Optica Spectrum Disorder (NMOSD), which are now recognized as separate entities with distinct pathogenesis. MicroRNAs are single-stranded small non-coding RNAs functioning in gene expression and protein synthesis and are emerging as novel diagnostic and prognostic biological markers. We identified differentially expressed miRNAs (DE-miRNAs) in patients with MS and NMOSD, and healthy controls.

Method

Serum small extracellular vesicles (EVs) from 12 patients with MS, 12 patients with anti-aquaporin-4-positive NMOSD, and 12 healthy controls (HC) were analyzed. We performed a comprehensive analysis of differentially expressed miRNAs (DE miRNAs) in serum EVs and identified miRNAs that exhibited significant differential expression between the groups using Nanostring analysis.

Result

We found several DE-miRNAs; 3 upregulated and 13 downregulated DE-miRNAs were observed in MS group and 10 upregulated and 22 downregulated DE-miRNAs were found in NMOSD group. Commonly in MS and NMOSD groups, miR-548a-5p was upregulated, but 5 miRNAs (miR-576-5p, miR-329-3p, miR-365b-5p, miR-507, miR-320b) were downregulated, compared to HC. In the comparison between MS and NMOSD, miR-6720-3p and miR-363-5p were significantly upregulated and 6 miRNAs (miR-1275, miR-1302, miR-33a-5p, miR-191-5p, miR-181b-5p+has-t, miR-514b-5p) were downregulated in NMOSD, compared to MS. We performed ROC analysis for all miRNAs, and based on high AUC values, we selected the top three miRNAs (miR-181b-5p, miR-191-5p, miR-6720-3p). When combining their expression data, we observed an improvement in ROC analysis performance, demonstrating the ability to distinguish between MS and NMOSD with high accuracy.

Conclusion

MS and NMOSD exhibit distinct miRNA profiles, suggesting that DE-miRNAs can potentially enhance the diagnostic accuracy of differentiating between these two disorders.

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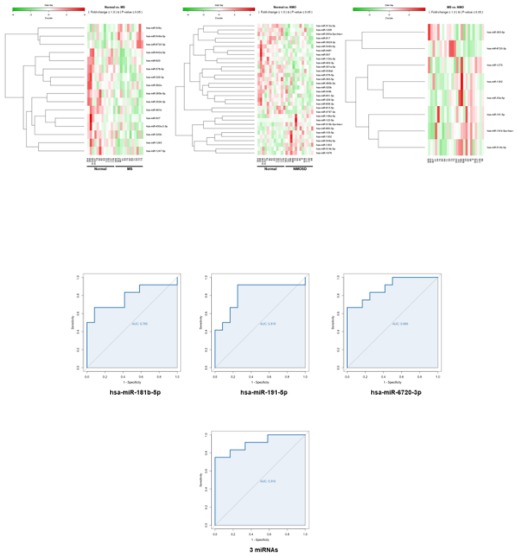

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Keyword: *microRNA, multiple sclerosis, Neuromyelitis optica spectrum disorder, extracellular vesicles*

#230 Tuberous sclerosis complex and epilepsy are associated with a pro-inflammatory peripheral immunophenotype and elevated levels of CNS inflammation and injury markers.

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Tuberous sclerosis complex (TSC) is a multisystem disorder caused by loss-of-function mutations in TSC1/2 proteins. These mutations lead to constitutive mTOR activation, altered cellular proliferation and differentiation and ultimately brain lesions such as tubers. Over 80% of TSC individuals have epilepsy, of which 60% become drug-resistant epilepsy (DRE). We and others have previously shown that in epilepsy, particularly DRE, levels of pro-inflammatory cytokines and biomarkers of neuroglial injury are elevated in peripheral blood. While the mTOR pathway is central to leukocyte biology, how TSC1/2 mutations affect the human peripheral immune system and whether inflammation contributes to the development of epilepsy and DRE remains unknown.

Using flow cytometry and multiplex immunoassays, we investigated the phenotype of peripheral blood mononuclear cells (PBMCs), levels of inflammatory cytokines, and markers of neuroinflammation in TSC patients.

Blood samples and clinical data were collected from TSC individuals (n=48, mean age of 36.1, 47.9% females), age and sex-matched healthy controls (HC, n=25), and non-TSC DRE (n=15). We found that individuals with TSC display significant

differences in the frequency and profile of PBMCs. We report that TSC individuals show a distinct serum inflammatory profile with altered levels of cytokines, chemokines, and growth factors such as CCL3, CXCL8 and EGF. Particularly, the proportion of B cells and the total IgG serum concentrations are increased in TSC compared to controls. In contrast, serum IL-7 levels and proportion of T cells are lower than in HC. Additionally, circulating monocytes were more frequent, whereas NK cells were reduced. We found that Th1 and Th17-related cytokines, such as IL-6, IL-17, IFNg and TNFa, and chemokines are increased in the serum of epileptic TSC individuals compared to non-epileptic TSC patients. Finally, we observed markedly elevated serum GFAP levels in TSC; both sNfL and GFAP were increased in TSC with epilepsy. Within the TSC patient groups, GFAP levels exhibited high inter-genotype variability. We validated the association between epilepsy and elevated GFAP and sNfL levels in a second independent cohort from the TSC Alliance Biosample Repository.

Overall, our results indicate that TSC is associated with altered distribution and profile of circulating leukocytes. Understanding the role of peripheral and CNS inflammatory processes in the development of neurological complications of TSC will provide insights into predicting and measuring responses to mTOR inhibitors as well as identifying new therapeutic targets in TSC and epilepsy.

Keyword: *Tuberous sclerosis complex, Epilepsy, biomarkers, neuroinflammation*

#249 Uncovering Molecular Signatures of the Dysregulated IFN System in Untreated MS and Reversal of Dysregulation by Manipulation with IFN Therapy.

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The dysregulated interferon (IFN) system in untreated multiple sclerosis (MS) causes severe immune dysregulation, yet preserves antiviral responses. The disrupted IFN system responds differently to IFN therapies such as IFN-beta-1b, IFN-beta-1a (subcutaneous and intramuscular) and the longer half-life, polyethylene glycol-conjugated IFN-beta-1a (PEG-IFN-beta-1a). Neurotrophic, immunoregulatory, and antiviral IFN therapy effects were measured in a pool of 529 patients with untreated MS, paired short-term and long-term IFN-treated relapsing remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS). The differential molecular signature of in vivo global RNA signatures of IFN-stimulated genes was obtained through Affymetrix HTA and Clariom RNA microarrays of MS peripheral blood mononuclear cells and in selected paired serum immune proteins with 65-plex and custom 11-plex Luminex multiplex arrays. Degree of expression was analyzed through correlational heatmaps, differentially activated pathways and biological processes were investigated. Long-term therapy primed the IFN system to overcome the subnormal responses in untreated MS. IFN-beta also balanced expression levels and pathways of immunoregulatory genes and proteins, with positive correlations between Th1 and Th2 biomarker families, correcting the dysregulated IFN system of untreated MS. PEG-IFN-beta-1a induced more neurotrophic genes and proteins than unconjugated IFNs. We also identify novel and important pathways in MS contributing to pathogenesis and development of new treatments.

Keyword: *Multiple Sclerosis, IFN therapy, Antiviral, Immunoregulation, Neurotrophic*

#257 Mechanism regulating stress granules dynamics in human oligodendrocytes

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Oligodendrocyte (OL) injury and subsequent loss is a pathologic hallmark of multiple sclerosis (MS). Stress granules (SGs) are membrane-less organelles containing mRNAs stalled in translation. Such formation has been linked with RNA binding proteins (RBPs) and is considered as participants of the cellular response to stress. Although SGs have been hypothesized to play a protective role, some studies suggest that the persistence of SGs may contribute to neuronal and glial damage. SG assembly and disassembly is a highly regulated process linked to mechanisms that regulate translation initiation including the integrated stress response (ISR) and the mechanistic target of rapamycin (mTOR) pathways. We have detected SGs in OLs in active and inactive areas of MS lesions as well as in normal-appearing white matter. Furthermore, we observed the involvement of the upstream translation-controlling pathways, the ISR and mTOR, in injured OLs in these tissues.

In our in vitro studies of human primary OLs, derived from surgically brain tissue specimens, we show distinct SG dynamics and molecular properties in response to acute stress (sodium arsenite, SA), chronic metabolic stress (glucose/nutrient deprivation) and inflammatory conditions (TNF-alpha, IFN-gamma). SA condition induces persistent SG formation that is inhibited by pharmacologic blockade (ISRIB) of the ISR regulatory pathway. We observe transient formation of SGs in hOLs exposed to metabolic stress conditions that are independent of the ISR pathway. Application of the mTOR inhibitor Torin1 results in persistent SG formation under both metabolic stress and control conditions. Pro-inflammatory cytokines themselves do not induce SG formation. However, combining these cytokines with metabolic stress conditions results in SG persistence in hOLs. We further demonstrate the dependence of SG persistence on the glycolytic metabolic properties of the OLs. Finally, we show that colocalization between different RBPs (PABP, hnRNP A1 and TDP-43) and SG markers is dependent on the nature and duration of the stress. Overall, our data suggest that persistence of SGs in OLs in MS reflects distinct changes in the protein translation regulatory pathways and glycolytic metabolic properties of these cells in response to a combination of metabolic stress and pro-inflammatory conditions.

Keyword: *Stress granules, Multiple sclerosis, Oligodendrocytes, Mechanistic target of rapamycin (mTOR), Integrated stress response (ISR)*

#260 Characterization of GPR160, as a novel putative immune biomarker of MS progression.

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Background: Multiple sclerosis (MS) is an autoimmune neurodegenerative disease in which the immune system invokes an attack on components of the myelin sheath. While currently, available treatments can manage the relapse-remitting type of MS (RRMS), between 30-60% of RRMS-affected individuals will ultimately develop a secondary progressive form of MS (SPMS) that is largely refractory to current drugs. Emerging evidence now implicates immune cell dysregulation in progressive forms of MS, and a key gap in the field is the paucity of readily identifiable markers that correlate with MS progression. We, therefore, hypothesized that studying alterations in the immune response in MS at different stages of the disease would reveal cell-specific targetable molecules in progressive disease.

Methods: We obtained Affymetrix gene chip data from PBMCs of 82 MS (18 RR, 64 SP) age-matched people and 29 healthy controls (HC) from the Accelerated Cure Project biorepository. We then designed a Bayesian machine-learning model, that used gene expression data, as well as key patient characteristics (sex, race, MS stage) to classify individuals with MS according to Expanded Disability Status Scale (EDSS) score. Putative gene expression biomarkers were independently validated using PBMC in the HITMS (Newfoundland) biorepository (18 MS, 18 HC) using qPCR. For murine analyses, we induced EAE by immunizing C57BL/6J mice with the encephalitogenic peptide $MOG_{[35-55]}$, followed by injection of pertussis toxin (PTX) on d0 and d2. Flowcytometric and qPCR analyses were conducted on ex-vivo isolated immune cells, as well as on differentiated $CD4^+$ T helper cells.

Results: The Bayesian model classified patients as having no clinical symptoms or signs; mild MS-related disability (EDSS <3), moderate disability (EDSS 3-6.5); or severe disability (EDSS ≥7), with area under curve (AUC)=0.976. These tertiles are standard in assigning disability categories in MS, based on Kalincik's definition of EDSS 3-6.5 as moderately advanced/advanced MS. Gpr160 was the transcript with the highest predictive value in the model, and we validated its expression, and positive correlation with EDSS, in the independent HITMS cohort. Ex vivo analysis of immune cells from MOG[35-55]-immunized revealed that Gpr160 transcript was augmented in $CD4^+$ T, $CD8^+$ T, B, and non-lymphoid CD45+ cells in the CNS as compared to the spleen. Interestingly, retrovirally-mediated overexpression of Gpr160 in myelin antigen-specific Th1 cells did not cause EAE of greater severity upon adoptive transfer as compared to control-transduced Th1 cells.

Conclusion: GPR160 expression is upregulated in peripheral blood immune cells of individuals with severe MS in two cohorts. Further, it is upregulated in CNS-infiltrating immune cells in the EAE model of MS. The specific immune cell distribution of GPR160 in humans, as well as the functional role of this protein in mouse CNS autoimmunity, are active areas of investigation in our lab.

Keyword: *Multiple sclerosis, EAE, T cell, GPR160, CNS autoimmunity*

#318 Extracellular Vesicles are increased in the blood of people with Radiologically Isolated Syndrome

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The radiologically isolated syndrome (RIS) describes a situation where a person without typical MS symptoms has a magnetic resonance

imaging (MRI) scan of their brain, which shows the characteristic lesions seen in people with MS (pwMS). Within five years, two-thirds of people with RIS (pwRIS) go on to develop new MRI lesions and/or clinical symptoms of MS. (1) Little is known about the disease mechanisms that predispose a pwRIS to develop MS symptoms. Extracellular Vesicles (EVs) are nanometer-sized particles that are released by cells and can contain ribonucleic acids (RNAs), proteins, and lipids. Previously, EVs were thought to be a means for cells to purge unnecessary molecules into the extracellular space; however, more recent evidence suggests that various cell types actively secrete them and that their cargo can be taken up by recipient cells. (2) Since EVs contribute to the transfer of biological material between cells under pathological conditions, RNAs and proteins derived from EVs circulating in the blood can mirror the altered state of the cell of origin. Small EVs (20-150 nm), sometimes called “exosomes”, are highly enriched in tetraspanins (e.g. CD9, CD63, and CD81), embedded in their lipid-bilayer membrane. Recently, it has been shown that blood-derived exosomes from pwMS can activate macrophages, (3) suggesting that exosomes transmit signals that can propagate immune cell activation in MS. Whether the number of EVs and their contents are altered in pwRIS is unclear. We use plasma from healthy individuals, pwRIS and pwMS and isolate EVs using size-exclusion chromatography (SEC). We then use nanoparticle tracking analysis (NTA) to quantify EVs and western blot (WB) to quantify tetraspanins in our preparations. We found increased EV numbers with NTA and increased teraspanin signals with WB in both pwRIS and pwMS compared to healthy individuals ($p<0.01$). Future studies will include an exploration of EV contents and if EVs propagate immune cell activation in RIS, as has been shown for MS. (3)

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Keyword: *Cell communication, Exosomes, Biomarker*

#357 Expression of bradykinin B2 receptor (B2R) in cerebrovascular inflammation models: a key for post-irradiation neuroinflammation imaging

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Brain metastases can be treated with radiosurgery, but unfortunately, 25% of patients develop chronic inflammation, which later leads to radionecrosis, impacting their quality of life irreversibly. Some evidence suggests that inflammation causes an overexpression of the bradykinin B2 receptor (B2R) by endothelial cells. Hence, B2R could be used as a biomarker to predict the onset of radionecrosis. Magnetic resonance imaging (MRI) in conjunction with micron-sized particles of iron oxide (MPIO) functionalized with anti-B2R antibodies (Ab) could detect B2R in vivo. As key first steps in preparing this new agent and establishing B2R as a biomarker, antibodies that bind B2R in our cell and animal models must be selected, and the

time course of B2R expression in our models must be established.

In vitro models:
a) Positive control cell line: Hek293 cells transfected with human B2R
b) bEND3 and RBMVEC (brain vascular endothelial cells, mouse, and rat respectively) in acute inflammation (incubated with lipopolysaccharide (LPS) or Interleukin 1-Beta (IL-1Beta))

Animal models: Fischer rat and balb/c mouse
a) Acute inflammation: intracerebral injection of LPS
b) Chronic inflammation and radionecrosis: single 90 Gy (at 100%) Gamma knife irradiation
c) Control (+): Rat F98 (Glioblastoma model)

Abs were qualified and tested with negative and positive samples (animal tissues and endothelial cells) for immunohistochemistry (IHC) and flow cytometry (FC). B2R levels were measured in vitro by FC and a bradykinin (BK) binding assay. B2R expression was assessed ex vivo by IHC in mice and rat tissues.
Five Ab were tested in FC and IHC. Only one was found to be adequate for IHC and FC. IHC results show a B2R level increase in vascular endothelial cells in inflammation conditions. However, the change in B2R levels may be difficult to assess in vivo as the expression increase is low in the FC and binding assays. Assessment of the mRNA expression of B2R is currently ongoing to further support our findings.

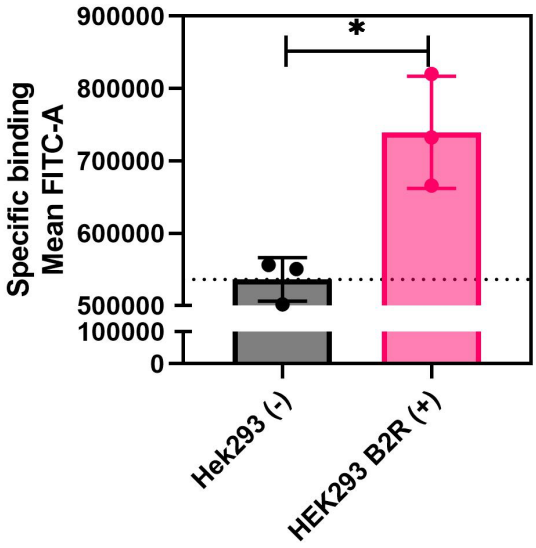

Figure 1. Flow cytometry with the control cell line. Validation of the affinity between B2R and the Ab used in further IHC and FC studies.

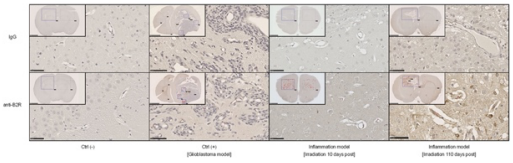

Figure 2. Immunohistochemistry on rat tissues. Comparison between non-specific binding (IgG) and B2R levels.
We determined that the affinity of most of the commercial Ab for B2R we tested was not sufficient for IHC or FC, and the only Ab that showed promise targeted the c-terminal part of B2R, which would be unsuitable for our application (targeting MPIO to B2R). Our results validate that B2R levels increase during inflammation. However, it is doubtful that B2R could be used as an imaging biomarker without developing a better targeting moiety that can target the n-terminal part of B2R.

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Keyword: *Radionecrosis, B2R, Inflammation, MPIOs*

#359 Investigating the neuroprotective mechanism of fumarate esters

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The intermediary metabolite fumarate is now recognized as an endogenous signaling mediator due to the ability to chemically modify protein cysteine residues when fumarate accumulates. This modification of protein thiols by fumarate is known as cysteine succination. In a similar manner, exogenously administered fumarate esters such as dimethyl fumarate (DMF) and diroximel fumarate (DRF) also result in the succination of protein cysteines to mediate their effects. For example, cysteine modification by DMF has previously been shown to upregulate Nuclear factor E2-related factor (Nrf2) transcriptional activity, driving a cytoprotective and immunomodulatory antioxidant response in models of multiple sclerosis (MS). The direct effects of DMF treatment in humans on select immune cell populations has been studied in detail, less is known about how these cell-permeable reactive fumarate esters may directly modify neurological targets to mediate beneficial neuroprotective effects.

In the present study we investigated novel neurological targets of DMF activity in the experimental autoimmune encephalomyelitis (EAE) murine model multiple sclerosis. The development of clinical symptoms was detected 11 days post-immunization, at which point the mice received either vehicle of DMF by oral gavage for 21 days. In the final 7 days of DMF therapy the mice had had significantly lower clinical scores ($P<0.001$) and had regained any initial body weight loss at the onset of the disease and therapy initiation. Following 21 days of DMF treatment the murine tissues were collected and we performed proteomic analysis of the spinal cord and brainstem to determine if DMF or its metabolite monomethyl fumarate (MMF) were directly succinating target proteins unique to the central nervous system (CNS). We detected fumarate-derived modification on ~30 different proteins, including robust succination of alpha-internexin on cysteine 24 (Cys24). Alpha internexin was the only succinated component of

CNS neurofilament proteins, despite prominent detection of other unmodified proteins such as neurofilament heavy chain. Further analysis of alpha-internexin protein levels demonstrated lower molecular weight cleaved forms in the vehicle treated EAE mice. In contrast, the alpha-internexin protein composition of DMF-treated EAE mice appeared similar to healthy control mice. The altered alpha-internexin protein profile detected in the EAE mice may be a novel marker of neurofilament damage, and our data suggests that cysteine modification on Cys24 by fumarate prevents further alpha-internexin cleavage, thereby preserving neuronal integrity in MS.

Influence of sex on neuroinflammation

#27 SEX DIFFERENCES IN THE EFFECT OF OBESITY ON ADAPTIVE IMMUNITY IN A MODEL OF MULTIPLE SCLEROSIS

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The causes of the initial autoimmune reaction in Multiple Sclerosis (MS) are unknown, but both genetic and environmental factors are thought to be involved. Female physiology increases MS risk by more than 3-fold and is one of the largest risk factors for developing this disease. Interestingly, the ratio of woman to men diagnosed with MS has been steadily increasing, suggesting that environmental factors are interacting with female sex to increase MS risk. One such factor could be adolescent obesity, which multiple epidemiological studies have found is strongly associated with development of MS primarily in females. Based on these observations, we put forward the central hypothesis that female sex and obesity interact to enhance central nervous system (CNS) autoimmunity. We explored the interaction of diet induced obesity (DIO) and sex on the development of CNS autoimmunity in the common animal model of MS, experimental autoimmune encephalomyelitis (EAE). We found that 4 weeks of high-fat diet (HFD) was sufficient to enhance T cell responses in both sexes with minimal metabolic impairment. We then found that male and female mice fed a HFD showed an increase in EAE disease severity compared to controls, but this effect was more pronounced in female mice. We observed that this was a result of an enhanced CD4+ IFN-gamma+ response that was selectively promoted in female mice by DIO and was due to an intrinsic enhancement of IFN-gamma production and signaling in female CD4+ T cells. Furthermore, we observed significantly increased serum levels of IFN-alpha only in the DIO-female mice. Loss of IFN-alpha receptor in CD4+ T cells removed the enhanced Th1 response caused by DIO and reduced EAE disease severity in female mice. These results highlight that an overweight state may interact with female sex to increase T cell IFN signaling through a novel sex-specific role of Type 1 IFN signaling in promoting T helper 1 immunity in obesity.

Keyword: *Multiple Sclerosis, Obesity, Sex differences, T cells, Interferon*

#43 The Female Bias in Experimental Autoimmune Encephalomyelitis (EAE): Sex Differences in the Efficiency of Antigen Presenting Cells

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One of the major risk factors for multiple sclerosis (MS) is female sex. Studies in MS and in experimental autoimmune encephalomyelitis (EAE), the animal model of MS, suggest that the increased susceptibility to central nervous system (CNS) autoimmunity relates to a more robust T helper (Th) 1 immunity in females. Consistent with this, past studies showed that female SJL mice immunized with myelin antigens in Complete Freund's adjuvant develop stronger Th1 responses than male mice, and that cells isolated from draining lymph nodes (dLNs) and spleens of female mice are more able to transfer EAE (passive EAE) compared to cells from male mice. Here we investigated whether controlling the Th1 or Th17 cytokine status of the donor myelin-reactive T cells, by skewing them to Th1 or Th17 would negate the sex difference in the ability of these cells to cause EAE. We observed that female donor myelin-reactive Th cells caused worse EAE regardless of Th cytokine status of the donor cells. This sex difference in passive EAE was due to a larger myelin-specific T cell pool in the spleens and draining lymph nodes of female donor mice; since equalizing the number of donor male and female myelin-reactive T cells negated the sex difference in passive EAE. Further studies in mice, revealed that naïve $CD4^+$ T cells from male and female mice showed no sex differences in the potential to proliferate to anti-CD3/anti-CD28 in vitro, however when similar numbers of female SJL myelin-specific T cell receptor (TCR) transgenic $CD4^+$ T cells were transferred into male or female recipient SJL mice that were then immunized with myelin antigen and CFA, the T cells proliferated more extensively in the female hosts. Flow cytometry analysis revealed a higher percentage and number of dendritic cells (DC) and higher expression of CD86 and IL-12p40, but not IL-10 by the DCs in the draining lymph nodes of the female hosts. Together, this work suggests that female mice are more able to prime myelin-specific T cells during immune responses that results in an overall increase in the size of the myelin-specific T cell pool and the potential of these cells to induce CNS autoimmunity.

Research funded by grants from the MS Society of Canada and the CIHR.

Keyword: *Multiple sclerosis, Experimental autoimmune encephalomyelitis, Sex differences, Dendritic cells, T helper cells*

#79 Increased biomarkers of immunosenescence in the peripheral blood of people with multiple sclerosis

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Introduction: Biological aging of the immune system, coined immunosenescence, leads to a progressive deterioration of the capacity to mount an appropriate robust immune response but with an increased predisposition to excessive levels of pro-inflammatory mediators. Aging is

associated with key changes in the physiopathology of multiple sclerosis (MS). As they age, people with MS (PwMS) experience more progressive forms of the disease and a lower efficiency and greater risks of adverse effects associated with disease-modifying therapies (DMTs). The mechanisms contributing to these events remain poorly understood. Since age, rather than disease duration, is more strongly associated with disease progression, we hypothesize that immunosenescence contributes to the age-related progression in MS.

Objectives: We aim to evaluate the age-associated biomarkers of immunosenescence in pwMS compared to age and sex-matched healthy controls (HC) and understand the impact of MS and of DMTs on age-related outcomes.

Methods: Blood samples and clinical data were longitudinally collected from PwMS over 1-3 years (n=611 samples) and age-sex matched HC (n=137). We characterized peripheral blood mononuclear cells (PBMC) using flow cytometry and bulk RNA sequencing and assessed multiple serum biomarkers using multiplex assays.

Results: Within the cohort, 10% of younger PwMS (<45y.o.) while >30% of the older ones (>45y.o.) had an EDSS ≥3. Our flow cytometry analysis demonstrated that the reduced proportion of naïve T cells expected in older individuals occurred early in untreated PwMS (<45y.o.). Paired analysis of longitudinal PwMS samples showed that the pwMS showed a stabilization of naïve to effector T lymphocytes ratio following DMT onset but decreased in pwMS who remained untreated. Moreover, PwMS displayed increased age-associated leukocytes changes compared to age and sex-matched HC, such as increased CD57+ T and B cells; DMT mitigated these changes. RNA sequencing of PBMCs confirmed the premature aging signature of circulating immune cells. Serum levels of IL-27, FGF, and EGF prior to therapy could distinguish first-line DMT responders from non-responders.

Conclusions: Our results underline that immunosenescence prematurely occurs in PwMS compared to age and sex-matched HC, and DMTs can attenuate specific age-related immune changes. A better understanding of the relationship between immunosenescence, disease progression, and DMTs effects will provide insightful information to improve disease management for the aging PwMS population.

Keyword: *Multiple Sclerosis, Immunosenescence, Aging, Inflammaging, Disease modifying therapies*

#85 Age-related alteration of fibroblast-like cell response to CNS injury

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Background: Wound healing is important for the regeneration of tissue architecture and function following injury. However, aberrant and chronic inflammation can dysregulate repair processes resulting in fibrosis. In multiple sclerosis (MS), repair and restoration of axon function in demyelinated central nervous system (CNS) lesions require remyelination by oligodendrocyte progenitor cells (OPC). However, aging impairs the repair process, leading to fibrotic scar formation that interferes with remyelination and functional recovery. While fibroblast-like cells (FLCs) from the meninges and perivascular space may enter the CNS parenchyma following injury and initiate fibrotic scarring, how FLCs impair remyelination and OPC function especially in the aging CNS remain unknown.

Objectives: We aim to assess the role of FLCs on remyelination, the impact of age on FLCs, and the mechanisms driving their age associated dysfunction.

Methods: We demyelinated the ventral spinal cord white matter of young (2-3-months) and middle-aged (12-months) mice through stereotaxic injection of lysolecithin (LPC). We assessed how age impacted OPC recruitment, maturation, and remyelination at days 7, 14, and 21 days post injury (dpi), respectively. Immunohistochemistry and confocal microscopy were used to identify platelet derived growth factor β (PDGFRβ) expressing FLCs in LPC lesions. CSPG4-Cre;Mapt-eGFP mice in which newly myelinating oligodendrocytes express eGFP were used to quantify remyelination. OPC-FLC co-culture experiments were used to determine how FLCs impacted OPC maturation. Transwell migration assays with Boyden chambers tested how bone-marrow derived macrophages (BMDMs) stimulated by different pro-inflammatory cytokines regulated FLC recruitment.

Results: FLCs persisted in the LPC lesion at all time points and the regions they occupied were devoid of Olig2+ oligodendrocyte lineage cells and GFP+ remyelinating cells. More importantly, both lesion volume and FLC occupied volume were increased in lesions of middle-aged mice and this was associated with an increase in extracellular matrix (ECM) deposition. These observations suggest FLCs impaired remyelination by OPC, and indeed, FLCs inhibited OPC maturation in co-culture. Since aberrant inflammation drives fibrosis, we compared the immune response in young and aging lesions. While there were less IBA1^{+} cells in the aging lesion, they appeared dysfunctional with elevated Arg1 and MerTK+CD206+ expression. Finally, using BMDM-FLC transwell assays, we found macrophages stimulated with IL-1β promoted FLC migration across the transwell.

Conclusions: FLCs impair OPC maturation and remyelination, and are more prevalent in the aging lesion environment. Aberrantly activated macrophages may facilitate FLC recruitment in the aging CNS.

Keyword: *Remyelination, Fibroblast, Aging, Ageing, Oligodendrocyte*

#98 Exposure to cigarette smoke creates a pro-inflammatory lung environment that alters the development of EAE

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Cigarette smoking (CS) increases the risk of multiple sclerosis (MS) and this effect is stronger in men than in women. By contrast, chewing tobacco protects against the development of MS, suggesting that it is the inhalation of smoke particles in the lung that is promoting T cell autoimmune mechanisms in MS. To understand how CS increases MS, we tested the effect of passive cigarette smoke exposure on the development of experimental autoimmune encephalomyelitis (EAE), a model of MS. We exposed C57BL6/J mice to CS or ambient air (AA) for 2 h per day/5 days per week for 8 weeks using a whole-body smoke exposure system. We then induced EAE by immunization with myelin oligodendrocyte glycoprotein (MOG) p35-55 and Complete Freund's adjuvant and pertussis toxin. We observed that CS enhanced MOG p35-55-

specific Th1 responses in the spleen. Paradoxically, CS decreased the severity of EAE, particularly in the male mice. Using a Th17 adoptive transfer model of EAE (AT-EAE) we observed that the protective effect of CS on EAE mapped to the recipient rather than the donor male mice; this correlated with a greater presence of MOG p35-55-specific T cells in the lungs and a reduced presence of these cells in the CNS. Male CS recipients also had a greater number of $CD45^+$ cells, B cells, eosinophils, innate lymphoid 2 type cells, and mature antigen presenting cells in the lungs compared to female CS or male or female AA groups. Thus, CS was creating a pro-inflammatory environment in the lung, particularly in males, that was detaining the myelin-reactive T cells on their way to the CNS. We hypothesized that this same biology could enhance EAE in a model where myelin-reactive T cells are primed in the lung. We therefore tested the effect of CS on EAE development in 2D2 TCR transgenic mice. We have found that male 2D2 mice exhibit a higher incidence of hindlimb clasping, a subclinical EAE sign, as compared to AA male 2D2 mice: only 1 mouse of 16 in the CS group developed classic EAE signs. Our findings suggest that CS may enhance CNS autoimmunity by increasing Th1 inflammation and drawing naive myelin-specific T cells into the lung where they have the potential to be activated by environmental factors.

Keyword: *multiple sclerosis, experimental autoimmune encephalomyelitis, cigarette smoking, lung inflammation*

#114 Sex-dependent effects of postnatal overfeeding on the development of central nervous system autoimmunity in mice

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Multiple sclerosis (**MS**) is an autoimmune disease of the brain and spinal cord that is characterized by demyelination and neuronal loss. While the exact cause of this disease remains elusive, major risk factors for MS have been identified, including female sex, adolescent obesity, and early pubertal onset. The goals of this project were to induce an overweight state in mice by postnatal overnutrition (**PNO**) and to characterize the effects of this intervention on the immune system and on the development of central nervous system (**CNS**) autoimmunity in a murine model of multiple sclerosis called experimental autoimmune encephalomyelitis (**EAE**). We hypothesized that PNO would exacerbate EAE in mice. PNO treatment was initiated in mice at 3 days of age by redistributing male and female pups to lactating dams such that litters were either controlled at N = 3 pups/dam (PNO group) or at N = 8 pups/dam (control group). Mice were cross-fostered with sex-matched pups. Compared to control, PNO treatment resulted in modest (10-20%) weight gain and accelerated pubertal onset in mice of both sexes (by 2-3 days). This weight gain with PNO was associated with greater fat mass, serum leptin levels and mild dyslipidemia in female mice only. Upon induction of EAE with myelin oligodendrocyte glycoprotein (MOG) p35-55/Complete Freund's adjuvant, PNO females exhibited an exacerbated EAE course compared to sex-matched controls. There were no differences in disease course between PNO and control males. Studies in a Th1 adoptive transfer model of EAE (AT-EAE) showed that MOG reactive T cells isolated from PNO donor mice induced an exacerbated from of EAE compared to control cells, indicating an effect of PNO on myelin-specific T cell priming or differentiation. Further studies revealed that

female PNO mice with EAE exhibited enhanced myelin specific T cell proliferation and IFN-gamma production compared to control mice upon restimulation with MOG_{35-55} ex vivo. Overall, these data suggests that early life adiposity can enhance murine susceptibility to autoimmunity, especially in females. Future studies will aim to explore the underlying immune- and sex-dependent mechanisms of this effect of PNO in EAE.

Keyword: *Obesity, Experimental Autoimmune Encephalomyelitis (EAE), Postnatal Overnutrition, Multiple Sclerosis (MS)*

#203 Sulforaphane treatment reduces pro-inflammatory cytokines and redox state in adult and old Wistar rats in a sexually dimorphic manner

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Increased neuroinflammation and oxidative stress have been associated with a higher risk of developing cognitive impairment and various neurodegenerative diseases during aging (1). It is known that some phytochemicals and dietary components display a typical hormetic response protecting the cells against several stressors (2). Sulforaphane (SFN) is an isothiocyanate that modifies the redox state and inhibits the activation of the transcription factor NFkB, thus it could decrease inflammation and oxidative stress associated with increasing age (3). The aging process is not homogeneous between species and sexes (4), therefore our aim was to evaluate if SFN long-term treatment was able to prevent age-associated neuroinflammation and oxidative stress in adult and old females and males Wistar rats. SFN was administered subcutaneously (0.5 mg/Kg, corresponding to 2.8 µM/Kg of body weight) 5 days per week for 3 months (5) in adults (12 to 15 months old), and old (18 to 21 months old) rats. Young rats (4 months old) were used as age controls. Fourteen cytokines and 8 chemokines were determined in the rat´s brain cortex (Cx) and the hippocampus (Hc) using the ProcartaPlex® Multiplex Immunoassay kit. Adult females showed higher levels of pro-inflammatory cytokines and chemokines in both brain regions than adult males, but no differences were observed between the aged groups. SFN was able to reduce this inflammation in female Cx and Hc during adulthood but not in de aged animals. Interestingly, higher levels of most cytokines and chemokines were found in the Hc than in Cx. GSH/GSSG ratio was determined as a redox state indicator (6). Both sexes' adult groups Cx showed lower oxidative levels when compared with their respective same-sex young. Furthermore, SFN-treated adult females obtained a higher GSH/GSSG ratio when compared with the same-age non-treated group, while no differences were observed against the young group. Our results showed that SFN treatment diminished pro-inflammatory molecules and redox state in a sexually dimorphic manner during adult age, but not during old age.

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Keyword: *Sulforaphane, aging, gender, neuroinflammation, dimorphism*

#220 Age related changes of systemic immune profile in nerve injured male and female mice

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Aging is associated with a higher prevalence of many chronic non-communicable diseases including chronic pain, and there is a higher prevalence of chronic pain in women than in men. Aging is also associated with low grade systemic chronic inflammation (inflammaging). However, the relationships between inflammaging, chronic pain, and sex difference have not been fully understood. We performed the spare nerve injury (SNI) and sham surgery on 3–4-month-old male and female mice (n=10/group), and longitudinally monitored them for 2 years. We used the von Frey and acetone tests to measure mechanical and cold sensitivities, and flow cytometry to determine immune cell compartments every 3 months. We evaluated changes in 111 serum cytokines and mediators at the ages of 4, 15, and 23 months using the Proteome Profiler XL cytokine assay. We also transferred 4-month-old (1-month post-SNI) male and female SNI serum to 3 month-old naïve male and female mice, then measured mechanical and cold sensitivity in the mice receiving the serum. We observed that both male and female SNI mice exhibited persistent, stable

mechanical and cold allodynia over the 2 years following surgery. Flow cytometry results showed age and sex dependent changes in circulating monocytes, NK cells, B cells, CD4 and CD8 T cells. However, the impact of nerve injury on the number of immune cells was minor. Proportionally, in aging female mice the monocyte and the neutrophil compartments expanded, while the lymphocyte compartment contracted. These proportional changes were not observed in aging male mice. Our cytokine/chemokine/growth factor proteome profiler results showed that in male sham mice, there was a robust age-related upregulation of serum mediators, which peaked at the age of 15-month-old. SNI surgery strongly magnified this pattern in male mice. In female mice, it appears that sham surgery resulted in a strong upregulation of serum mediators, maintained relatively stable over 2 years. However, the SNI did not lead to such upregulation in female mice, and most factors displayed slight up- or down-regulation. No overt age-associated changes were observed. Interestingly, serum from both male and female 1 month-SNI mice triggered painful behavior in naïve mice. Unexpectedly, the impact of female SNI serum was more intense and lasted longer than that of male SNI serum. To conclude, our results show that there is a sex dimorphism in the longitudinal development of systemic inflammation with age and following nerve injury. While serum mediators examined in the current study may explain the systemic contribution of pain in male mice, the lack of upregulation in serum mediators in female SNI mice suggests an alternative mechanism for the systemic contribution to pain. Further investigation into systemic mechanisms of pain and inflammaging will help us to better understand the mechanisms of how chronic pain is maintained, and to develop effective pain management strategies in the future.

Keyword: *Inflammaging, Neuropathic pain, Sex difference, Systemic inflammation*

#221 Male sex determines long-term neuroprotection of an Interleukin 17A antibody treatment after stroke

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Objectives:

The activation of the immune system has a profound impact on the fate of the ischemic brain tissue and neurological outcome in experimental stroke. However, the translation from preclinical animal studies into the clinics has failed so far. Reasons for this include differences in study designs between preclinical and clinical trials as preclinical studies mostly use young male animals, and shorter observation periods.
Among the inflammatory cascades, which drive the early excessive sterile immune response, Interleukin 17 (IL-17) holds an important role. In ischemic hemispheres, IL-17 is rapidly produced by atypical T cells and amplifies the early detrimental inflammatory response through the upregulation of neutrophil attracting chemokines. The aims of our study were to test whether sex and age influence the expression and the effects of IL-17 following ischemia and if an IL-17 neutralization improves long-term outcome in aged mice of both sexes.

Methods:

As the gut-microbiota and the generation of short-chain fatty acids (SCFA) strongly influence IL-17 production in T cells we performed microbiota 16sRNA sequencing and measured the IL-17 levels under steady state conditions in mice of differing age and sex.
We employed the transient middle cerebral artery occlusion (tMCAO) model to investigate

the cellular inflammatory response and IL-17 levels in ischemic brains and the peripheral immune compartment by flow cytometry (FACS). To study the effects of an early IL-17 neutralization on long-term outcome we treated aged male and female mice with an IL-17Antibody (Ab) vs IgG-control within 6 hours after tMCAO and analyzed lesion volume, behavioral outcome, and the inflammatory response by FACS and immunohistochemistry up to 3 months after stroke.

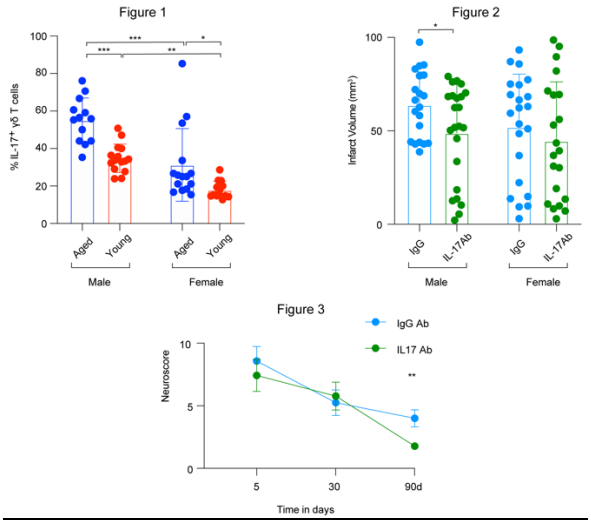
Results:

16sRNA sequencing reveals a distinct footprint of the microbiota of aged compared to young mice under baseline conditions which goes in hand with reduced SCFA producers. Furthermore, the analysis of the IL-17 production in γδ T cells shows that aged male mice exhibit significantly higher IL-17 levels compared to young and aged female mice which is preserved after stroke (Figure 1).

Neutralization of IL-17 6h post-tMCAO only reduces the infarct volume significantly in aged male but not in female mice on day 3 after stroke (Figure 2). Notably 90 days after stroke only aged male mice show significantly reduced mortality and improved long-term functional outcome after an IL-17Ab treatment whereas there is no significant difference in aged females (Figure 3).

Conclusion:

We can show that neutralization of IL-17 leads to improved neurological outcome in aged male mice over a period of 3 months, whereas old females are not protected underlining the therapeutic potential of an IL-17 neutralization in stroke. Consequently, sex differences and aging should be included to develop an efficient immunotherapy for stroke patients.



#241 Sex Differences in a Key Regulatory T Cell Pathway with Implications for Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease affecting the central nervous system (CNS), characterized by demyelination, axonal damage, and neuronal degeneration. Inflammatory autoimmune diseases exhibit a gender gap, such as multiple sclerosis, which shows a higher prevalence in females than males. The sex bias in MS emerges post-puberty strongly implicating a role for gonadal hormones in disease risk.

T cells has been considered to drive MS. CD46, a co-stimulatory protein, triggers T lymphocyte activation, leading to the conversion of Th1 cells

into Type 1 regulatory T cells (Tr1). This pathway of Tr1 activation is impaired in MS, with patients exhibiting reduced interleukin-10 (IL-10) production by CD46-co-stimulated T cells. Our unpublished data further indicate a positive interaction of CD46 with activity of another co-stimulatory marker CD226 in Tr1 generation and IL-10 production. Whether there is a sex difference in the effects of CD46 and CD226 co-stimulation in Tr1 generation remain unclear.

In this context, we conducted in vitro experiments using primary CD4+ T cells obtained from male and female healthy donors. These cells were activated with anti-CD3 and co-stimulated with either CD46 or with anti-CD46 and anti-CD226 activating antibodies. We observed a sex difference in Tr1 generation under both activation conditions. Indeed, female Tr1 cells exhibited lower production of IL-10 and IFN-gamma compared to male Tr1. The lower cytokine response in the female donors did not relate to a defect in T cell activation. Upon CD46/CD226 co-stimulation, Tr1 cell generation proceeded just as efficiently in the male and female donor T cells. However, the generated Tr1 cells from females had a lower propensity to secrete IL-10. Thus, the interaction of CD46 and CD226 enhances the anti-inflammatory activity of Tr1 cells only in the male donors.

Currently, we are investigating these pathways in male and female patients with relapsing-remitting MS and are studying the underlying basis for these sex-based differences. Collectively, our findings suggest that there is a sex bias in a crucial regulatory pathway that regulates T cell homeostasis that is defective in MS.

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Keyword: *Multiple sclerosis, CD46, CD226, Type 1 regulatory T cells, Sex bias*

#247 Female sex is associated with higher risk of experiencing MS reactivation after Fingolimod cessation

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Background: Data on sex differences in Multiple Sclerosis (MS) therapy safety are limited despite their clinical significance. Fingolimod (FTY), the first-in-class Sphingosine 1-Phosphate Receptor (S1PR) Modulator (S1PRM), is a treatment for MS that targets S1PRs on lymphocyte surfaces, reducing their exit from lymph nodes. Upon treatment cessation, up to 10% of patients experience a relapse, sometimes referred to as disease reactivation. Our study aims to investigate the sex-specific aspects of disease activity following FTY cessation.

Methods: We used the MS mouse model myelin oligodendrocyte glycoprotein peptide 35-55 experimental autoimmune encephalomyelitis (MOG_{35-55} EAE). Disease reactivation was defined as an increase of >= 2 EAE points after FTY stop. Wild-type C57BL/6JRj mice of both sexes were treated orally with FTY (0.1 mg/kg) dissolved in condensed milk or vehicle control for 20 days from immunization. The experiment ended on day 28. Disease severity was monitored daily according to a 10-point scale. S1PR1 expression on T cell was assessed by immunohistochemistry on spinal cord sections. A literature review and analysis of two open registries (FDA Adverse Event Reporting System, EudraVigilance) for adverse events were conducted to determine the incidence of MS disease reactivation after FTY stop in regard to sex.

Results: In vivo, we observed a higher cumulative disease score in female compared to male mice (p-value<0.001) in the post-treatment period. The expression of membrane S1PR1 by CD3+ T cells infiltrating the central nervous system increased after treatment in female mice treated with FTY, while no such change was observed in male mice (p-value<0.0001). From the literature review, seven cohorts were included with in total 2'465 patients (1'799 women, 666 men) who discontinued FTY. Among them, 307 patients (246 women, 61 men) experienced disease reactivation. Female sex was associated with a higher risk of disease reactivation (odds ratio [OR] 1.57, 95% confidence interval [CI] 1.17-2.11, p-value<0.01) after treatment cessation.

Conclusion: Our investigation of different patient cohorts from the literature and open registries revealed an association between a higher risk of disease reactivation after FTY therapy cessation and female sex. The reasons for sex differences remain unclear; a possible mechanism could be the differential expression of S1PR1 by T cells, with higher receptor expression in female mice.

Keyword: *sex difference, fingolimod, multiple sclerosis, disease reactivation*

#302 Sex hormone correlations with inflammatory biomarkers, metabolites, and short chain fatty acids in women with multiple sclerosis.

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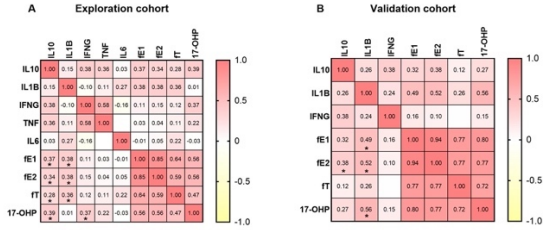

Fig. 1AB: Heatmaps showing spearman correlation coefficients (rho) between inflammatory markers and sex hormones in the exploratory cohort (A) and validation cohort (B). Significant results are marked with *.

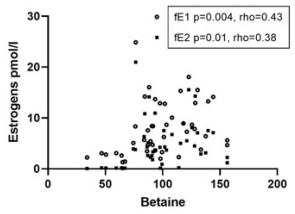

Fig. 2: Free estradiol (E2) and estrone (E1) spearman correlation with the blood metabolite betaine in 44 women with MS.

Introduction

The mechanisms behind the sex-biased occurrence of multiple sclerosis (MS) with twice as many women affected by the disease is still largely unknown. The cyclic regulation of sex hormones in women could affect peripheral

blood biomarkers to increase susceptible to MS and cause higher disease activity levels, as recently reported (1). Here we have measured sex hormones in women with MS to explore their relationship with biomarkers of inflammation, metabolites, and short chain fatty acids (SCFA), all measured in the same blood sample.

Methods

Blood was sampled in two cohorts of women with relapsing-remitting MS before their first disease modifying treatment, as previously reported (2). Sex hormones were measured on serum LC-MS/MS and sex hormone binding globulin (SHBG) was measured with a chemiluminescent immunoassay. Inflammation biomarkers, metabolites, and SCFA were measured, as previously described (3), the last two in a subgroup of 44 women.

Results

We measured estradiol (E2), estrone (E1), testosterone (T) and 17- hydroxyprogesterone (17-OHP) in 55 women with MS and the free unbound concentrations of E1, E2 and T was calculated using SHBG. Sex hormones were correlated with mRNA expression levels of proinflammatory and regulatory cytokines and showed significant correlations ($p<0.05$) with interleukin 10 (IL10) interleukin 1B (IL1B) and interferon-gamma, the last only with 17-OHP (Fig.1A). In an independent cohort of 28 women with MS we validated correlations of IL10 with fE2 and IL1B with fE1, fE2 and 17-OHP (Fig.1B) at the protein level. In a subgroup of 44 women SCFA did not correlate significantly with sex hormones ($p<0.01$) and only the blood metabolites betaine and phosphoethanolamine (PE) correlated significantly ($p<0.01$) with fE1, fE2 and fT (Fig.2).

Discussion

We find here that key components of the inflammatory response, IL10 and IL1B, follow the cyclic regulation of sex hormones in women with MS, at both mRNA and protein level. PE is used for the endometrial tissue thickening in the luteal phase of the menstrual cycle (4), which could explain the significant correlation with sex hormones. Betaine, a known methyl-donor, has been shown to protect against axonal damage in a mouse model of MS (5). Further validation of betaine will be needed and future serial measures during the menstrual cycle could clarify a possible interplay between betaine, IL10, IL1B and sex hormones.

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Keyword: *IL10, IL1B, Betaine, SCFA, sex hormones.*

#335 Sex chromosomes and sex hormones play opposing roles in regulating the severity of Th17 cell-mediated disease in a model of chronic CNS autoimmunity

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While women have a higher incidence of relapse/onset MS, men are more prone to developing progressive forms of MS. We are investigating the underlying reasons of this difference using a Th17 cell-mediated mouse model of chronic progressive experimental autoimmune encephalomyelitis (EAE) established in our lab. We adoptively transfer

Th17 cells from 1C6 mice, which bear a transgenic TCR directed against MOG[35-55] autoantigenic peptide, to NOD.Scid (NS) mice. We recently found that male Th17 cells induce disease of greater severity than female Th17 cells. To dissect the role of sex hormones vs chromosomes, we are now exploit four-core genotyope (FCG) double-transgenic mice, in which the male sex determining gene Sry is knocked out of the Y chromosome (YSryKO) and is simultaneously knocked in to autosomal Chr 3 (Tg-Sry). When TgSryXYSryKO males are crossed to a WT female 1C6, we obtain two hormonally male strains (Tg-SryXX:1C6 and TgSryXYSryKO:1C6) and two female strains (XX:1C6 and XYSryKO:1C6). Adoptive transfer of Th17 cells derived from each of these genotypes reveals that the presence of male chromosomes exacerbates EAE severity while the presence of male hormones ameliorates it. When donor Th17 cells were analyzed, we found that Sry+ mice (TgSry XX:1C6 and TgSryXY-:1C6) were having higher expression of Androgen receptors and lesser expression of Th17 master transcription factor (RORγt compared to Sry- mice (XX;1C6 and XY-:1C6). Intriguingly, in another set of experiment, it was found that Th17 cells from Sry-XX were significantly more pathogenic compared to WT 1C6 female mice despite being. Gonadectomizing of Sry- XX mice did not diminish the pathogenesis of Th17 cells derived from these mice, pointing towards a possible effect of epigenetic imprinting in these mice. Together, these data reveal an elegant and multi-layered contribution of biological sex variables to the pathogenesis of Th17 cells in a model of CNS autoimmunity.

Keyword: *Multiple Sclerosis, EAE, Th17 cells, Sex difference*

Involvement of glial cells in neuroinflammation

#38 Cystatin C expression in astrocytes contributes to disease pathogenesis in female but not male mice with experimental allergic encephalomyelitis.

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Cystatin C (CysC) is a protease inhibitor that is increased in the brains of people with multiple sclerosis (MS), as well as the central nervous system (CNS) of mice with experimental allergic encephalomyelitis (EAE) – a model of MS. We previously showed that CysC, which is expressed by immune cells and CNS cells such as astrocytes, plays a detrimental role in EAE by promoting antigen presentation, but only in female animals. We sought to elucidate if the negative effect of CysC in EAE is contributed by its presence in astrocytes. Using a CysC-astrocyte conditional knockout mouse ($CysC^{fl/fl}$/GFAP-Cre^{+}/TdT^{+} or CysC-CKO) that we created, we found that clinical disease was markedly reduced in female CysC-CKO EAE animals relative to controls but there was no difference among the male genotypes. The reduced EAE disease in female $CysC^{fl/fl}$/GFAP-Cre^{+}/TdT^{+} mice was accompanied by significantly reduced demyelination as measure by eriochrome cyanine staining, an absence of CNS infiltrating immune cells (CD45, CD3, CD4 and CD8) and minimal IgG staining compared to peak female WT EAE controls. Since these results suggested that blood brain barrier permeability may be compromised, we assessed for tight junction protein expression (Occludin, ZO-1) using immunohistochemistry. Qualitative analyses indicates that there is more ZO-1 and Occludin staining in female CysC-CKO mice compared to their control counterparts. Quantification and live imaging will verify this

observation. Future experiments will assess if CysC impacts the activation, survival and phenotype of astrocytes. Altogether, our results indicate that CysC expression in astrocytes may contribute to the detrimental role of this protease inhibitor in EAE by promoting blood brain barrier permeability and thus entry of immune cells into the CNS. Intriguingly, this only occurs in female animals indicating that CysC has a negative sex dependent role in astrocytes.

YZ is supported by a Multiple Sclerosis Canada endMS Doctoral Studentship Award

Keyword: *Multiple Sclerosis, Cystatin C, Astrocytes, Experimental Allergic Encephalomyelitis*

#46 Age delays microglia response in LPC-induced demyelination

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Multiple sclerosis (MS) is a chronic inflammatory disease characterized by central nervous system (CNS) lesions, resulting in axonal loss and physical and cognitive disability. Regeneration of myelin sheath, known as remyelination, protects axons from degeneration, thereby slowing the permanent disability related to axonal loss. Remyelination declines during aging. However, it is still unknown what causes this age-dependent decline. Given that aging is associated with increased reactive oxygen species (ROS) in the CNS, we hypothesize that a population(s) of microglia/macrophages in the CNS produce excess ROS contributing to age-associated remyelination decline. We used the LPC (lysophosphatidylcholine) model of demyelination and examined the presence of age-associated ROS production during remyelination in young (2-3months) and middle-aged (8-10 months) mice receiving intraspinal LPC injections. We first characterized the accumulation of microglia and monocyte-derived macrophages using microglial fate-mapping with CX3CR1CreEr; RosatdTom mice. Age is associated with a delayed accumulation of microglia but not monocyte-derived macrophages. The delay in microglial accumulation may contribute to age-dependent remyelination decline. To understand the relationship between microglial accumulation and reactive oxygen species (ROS) deposition, we quantified malondialdehyde(MDA) within remyelinating lesions. We found that ROS deposition and microglia accumulation peak in young mice at an early stage of remyelination, coinciding with debris clearance. By contrast, with middle-aged mice, peak ROS deposition is delayed, suggesting that both ROS deposition and microglia accumulation are delayed in middle-aged mice. Understanding the relationship between microglia and ROS may provide new approaches to promote remyelination and protect white matter.

Keyword: *Multiple Sclerosis, Reactive Oxygen Species, Aging, Remyelination*

#68 The role of ICAM-1 in oligodendrocyte-immune cell interactions in neuroinflammation

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Multiple sclerosis (MS) is an immune-mediated inflammatory and neurodegenerative disease of the central nervous system in which myelin-

producing oligodendrocytes (OLs) are damaged, resulting in demyelination and compromised neuronal integrity. Neuroinflammatory processes are known to be modulated by pro-inflammatory CD4 T cells in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). Pro-inflammatory CD4 Th17 cells can establish direct stable contacts with OLs during EAE and exert cytotoxicity towards human OLs; however, the exact molecules involved in T cell-OL interactions are unknown. Upon exposure to inflammatory cytokines or to activated T cells, human OLs upregulate intercellular adhesion molecule 1 (ICAM-1), suggesting that this molecule may play a role in OL damage in pathological conditions. We hypothesize that interactions between ICAM-1 on OLs and its corresponding ligand, LFA-1, on CD4 T cells contribute to direct cell to cell contacts and subsequent T cell-mediated OL injury. Pharmacological blockade of ICAM-1 on human primary OLs or of LFA-1 on Th17-polarized cells using recombinant human ICAM-1 prior to co-culture resulted in reduced OL apoptosis in vitro, suggesting that ICAM-1/LFA-1 interaction is detrimental for OLs. In addition, the reduced ensheathment length of inflamed OLs upon co-culture with Th17 cells was prevented with pharmacological blockade of ICAM-1 on inflamed OLs. In contrast, OL-specific ICAM-1 knockout ($PLPcre^{ERT2}$:$ICAM\text{-}1^{fl/fl}$ mice) does not affect EAE clinical course in the chronic phase but is associated with an earlier EAE onset compared to controls, suggesting that oligodendrial ICAM-1 may be protective, potentially through its soluble form. Our data suggests that different mechanisms likely exist to regulate ICAM-1 dependent interactions between OLs and T cells in mice and human OLs. Overall, this work contributes to the understanding of mechanisms underlying T cell-mediated OL damage and points to a beneficial role of soluble ICAM-1 in neuroinflammatory conditions.

Keyword: *multiple sclerosis, experimental autoimmune encephalomyelitis, oligodendrocytes, T cells, ICAM-1*

#81 The impact of oxygen levels on inflammatory astrocyte functions

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Multiple sclerosis (MS) is a debilitating neuroinflammatory condition characterized by chronic inflammation, demyelination, and axonal damage within the central nervous system (CNS) (1). Astrocytes, the most abundant glial cells in the CNS, play crucial roles in CNS homeostasis (1). In addition, when activated, astrocytes can produce inflammatory factors that are toxic to neighboring cells and activate immune cells, thereby contributing to the perpetuation of inflammation and neuronal damage observed in MS (1).

Conventional techniques used to culture astrocytes expose these cells to substantially higher oxygen levels (21%) than those found in the brain (3-6%) (2). Moreover, a subgroup of MS patients exhibits even lower brain oxygen levels than normal (hypoxia) (3). Since astrocytes are potent sensors of oxygenation (4), oxygen conditions might play an important, yet understudied, role in regulating their homeostasis, reactivity, and immunological properties.

In this study, we aimed to elucidate the impact of oxygen levels on astrocyte homeostasis, reactivity, and immune functions. We cultured murine astrocytes at various oxygen levels, mimicking atmospheric (21%), physiological (4.5%), and hypoxic (1%) environments. We then assessed the effect of these oxygen levels on astrocyte homeostasis and their response to inflammatory stimuli, such as lipopolysaccharide (LPS) and a combination of IL-1alpha, TNF-alpha

and C1q. We show that, compared to atmospheric conditions, physiological oxygen levels reduce the proliferation of astrocytes both constitutively and in response to a scratch insult. Moreover, using flow cytometry we found that physiological oxygen levels decrease the expression of inflammatory astrocyte markers. Furthermore, proteomic and multiplex cytokine analysis revealed that hypoxic conditions alter vesicle transport pathways and cytokine secretion profiles of cultured astrocytes.
Together, our data set the stage for gaining a deeper understanding of the influence of oxygen levels on astrocyte homeostasis and reactivity, as well as provide novel insights into the role of hypoxia in MS pathophysiology. These findings may pave the way for the development of innovative therapeutic strategies targeting astrocyte reactivity and/or hypoxia in the treatment of MS.

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Keyword: *Astrocytes, Multiple sclerosis, neuroinflammation, oxygen levels*

#87 Microglia and neurons in the developing neonatal mouse cortex show distinct transcriptional and translational signatures following the lipopolysaccharide mediated innate immune challenge

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Neonatal mouse brain is very dynamic, with activated microglia involved in the processes such as synaptic pruning, immuno-surveillance and homeostasis. An immunological insult during the early developmental stages can lead to aberrant microglial response, ultimately damaging the neurons and potentially leading to neurological disorders. While studies have been focusing on the identification and description of context-dependent microglia immune transcripts, in vivo microglial and neuronal proteomics and associated regulatory mechanisms in neonates are less well defined (Beutner et al, Glia, 2013). One of the limiting factors is lack of appropriate animal models to study real-time in vivo transcriptional and translational dynamics. To decipher the microglial-neuronal molecular communication in vivo, we created the transgenic CD11brGFPxNFLrRFP mouse line, where the ribosomes of microglia are labelled with GFP and FLAG and of neurons with RFP. We performed a systemic lipopolysaccharide injection on unsexed post-natal day (P) 9 mice to stimulate immune response and analyzed on P10 (n=6, saline as control). Using modified translational ribosome affinity purification, we took a snapshot of the dynamic translational state of microglial and

neuronal ribosomes by capturing the real-time transcribed mRNAs and translated peptides. The collected RNAs were subjected to Affymetrix® mouse gene chip array and the peptides to mass spectrometry. We identified the top mRNA and peptide signatures associated with microglia and neurons. Interestingly, we found that the top upregulated microglial and neuronal transcripts were not translated. Both the microglial and neuronal mRNA signatures suggest an inflammatory profile, but their peptidyl profiles gravitate towards homeostasis. We verified our findings by performing western blot on microglia and neurons which were isolated using CD11b beads and the neuronal isolation kit (Miltenyi Biotec®). Additionally, our results from microglial cytokine array indicate no distinct differences between the expression of inflammatory cytokines between the LPS-treated male and female neonatal mice. Collectively our results indicate a discrepancy in the mRNA and the peptide profiles of microglia and neurons in neonatal mice post the LPS-challenge. This possibly hints at the existence of a post-transcriptional regulation. Targeting such regulators may normalize the immune profile by aiding microglial phagocytosis and ultimately bringing homeostasis, thus paving a way to novel therapeutic targets.

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Keyword: *microglia, transcriptomics, inflammation, neonates*

#94 Spatial cell type mapping of subcortical white matter lesions in multiple sclerosis

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Multiple sclerosis (MS) is a prototypic chronic-inflammatory disease of the central nervous system. After initial lesion formation during active demyelination, inflammation is gradually compartmentalized and restricted to specific tissue areas such as the lesion rim in chronic

active lesions. However, the cell typespecific and spatially restricted drivers of chronic tissue damage and lesion expansion are not well understood. We sought to better understand tissue compartmentalization and interaction of different cell types among different white matter lesion types in MS, by constructing a spatial map of gene expression for each cell type at various inflammatory stages of lesion pathology. We characterized subcortical MS lesions according to the level of inflammatory activity as acute (MS-A), chronic active (MS-CA), and chronic inactive (MS-CI) based on histopathology. Subsequently, we conducted single-nucleus RNA sequencing (n=19) combined with paired spatial transcriptomics (n=22) to integrate and analyze gene expression based on both modalities. Our analysis focused on decoding cell subtype diversity, identifying cell-cell communication patterns and signaling signatures across both lesion and non-lesion tissue areas in MS. The findings were further validated using immunohistochemistry and multiplex RNA in situ hybridization techniques.We could characterize various homeostatic and reactive MS cell types (n=11) within different lesion types and their various cell states, along with their spatial positioning within each lesion. Specifically, we identified various proinflammatory myeloid cell and astrocyte subtype signatures localized to MS lesion rim areas, highlighting iron, TNF and TREM2-APOE signaling among other MS-specific pathways. Furthermore, we identified an MS-specific astrocyte subtype characterized by the expression of ciliaassociated genes within MS lesion areas.

#97 Role of astroglial TNFR2 signaling in synaptic and cognitive function in multiple sclerosis

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In addition to debilitating sensory-motor impairments, the majority of individuals with multiple sclerosis (MS) suffer from a wide range of cognitive deficits. MS-associated cognitive dysfunction has been linked to disrupted synaptic transmission and plasticity, which require the bidirectional communication between neurons and astrocytes. A key modulator of synaptic plasticity is tumor necrosis factor (TNF), which signals via two receptors, TNFR1 and TNFR2, both expressed in astrocytes. Astroglial TNFR1 is essential for proper physiologic synaptic plasticity, but in the experimental autoimmune encephalomyelitis (EAE) model of MS activation of astroglial TNFR1 causes changes in excitatory synapses that lead to learning and memory deficits, indicating that overactivation of this receptor in disease turns detrimental. Remarkably, nothing is known about the contribution of astroglial TNFR2 to synaptic plasticity and cognition in MS, despite the fact that previous studies indicated a protective function of TNFR2 signaling in both MS and EAE. Our aim in this study is to address this gap of knowledge.

To investigate the role of astroglial TNFR2 in synaptic plasticity and cognition, we generated two mouse models with inducible, astrocyte-

specific ablation (GFAPcre^{ERT2}:TNFR2$^{fl/fl}$ mice) and overexpression (GFAPtTA:TRE-TNFR2 mice) of TNFR2, respectively. In physiological conditions, GFAPcre^{ERT2}:TNFR2$^{fl/fl}$ mice with astroglial TNFR2 ablation displayed impaired hippocampal long-term potentiation (LTP) and cognition. Following EAE, GFAPcre^{ERT2}:TNFR2$^{fl/fl}$ mice showed worsening of cognitive deficits compared to control TNFR2$^{fl/fl}$ mice at chronic disease, and this was accompanied by altered expression of hippocampal pre-synaptic proteins (e.g. SNAP25, synaptotagmin1/2) and glutamate receptors (e.g. GluR2/3). On the contrary, GFAPtTA:TRE-TNFR2 mice with astroglial TNFR2 overexpression showed improvement in cognitive function compared to their respective controls at chronic EAE. Collectively, these data indicate that astroglial TNFR2 is needed not only for proper synaptic function in physiological conditions, but also to contain and counteract synaptic and cognitive dysfunction associated with neuroimmune disease. This supports the concept that TNFR2 may be a therapeutic target in MS.

Keyword: *Multiple Sclerosis, Glia, Cognition, Synaptic Function*

#115 Microglial aggregation and demyelinating cortical pathology in mouse

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Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS) in young adults, with an autoimmune etiology. Currently approved therapies for MS are most effective at inflammatory stages of disease. The goal of our research is to establish an animal model for progressive MS (PMS).

Subpial cortical lesions and grey matter pathology with minimal blood-brain barrier disruption are hallmarks of PMS, which are not well modelled by current animal models. Our aim is to create meningeal inflammation, with cortical inflammation with activated microglia/macrophages, in order to understand the role of microglia in the chronic lesion and the underlying pathological mechanisms.

We induced cortical inflammation by feeding the myelin toxin cuprizone to mice. Preliminary data showed small aggregates of activated Iba-1+ microglia with accompanying demyelination in cortical grey matter. These aggregates were intensified by subarachnoidal injection of cytokines and by intrathecal injections of lipopolysaccharide or neurodegeneration-primed microglia. We are optimizing an alternative approach of laser irradiation with skull thinning, that induces a similar focal subpial aggregate of activated microglia. The volume of the irradiation-induced lesion reduced by 5 days post-irradiation. However, prior intrathecal administration of a viral vector expressing interferon-gamma led to sustained microglial activation at 5 days post-irradiation. Flow cytometry showed increased numbers of CD11c+ microglia, as well as monocytes and myeloid cells, at 5 days compared to the contralateral unirradiated hemisphere.

We will further optimize and establish these novel animal models that mimic the hallmark pathology of PMS and investigate the underlying pathological mechanism and kinetics. This will improve our understanding of, and lead to better treatment of PMS.

Keyword: *MS, cortical lesions, microglia, astrocytes*

#132 Oligodendrocyte progenitor cells differentiation induction with MAPK/ERK inhibitor fails to support repair processes in the chronically demyelinated CNS

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Remyelination failure is considered a major obstacle in treating chronic-progressive Multiple Sclerosis (MS). Studies have shown blockage in the differentiation of resident oligodendrocyte progenitor cells (OPC) into myelin-forming cells, suggesting that pushing OPC into a differentiation program might be sufficient to overcome remyelination failure. Others stressed the need for a permissive environment to allow proper activation, migration, and differentiation of OPC. PD0325901, a MAPK/ERK inhibitor, was previously shown to induce OPC differentiation, non-specific immunosuppression, and a significant therapeutic effect in acute demyelinating MS models. We examined PD0325901 effects in the chronically-inflamed central nervous system. Treatment with PD0325901 induced OPC differentiation into mature oligodendrocytes with high morphological complexity in vitro. However, treatment of Biozzi mice with chronic-progressive EAE, which reproduces major features of chronic MS, with PD0325901 showed no clinical improvement in comparison to control group, no reduction in demyelination, nor induction of OPC migration into foci of demyelination. PD0325901 induced a direct general immunosuppressive effect on various cell populations, leading to diminished phagocytic capability of microglia and less activation of lymph-node cells. It also significantly impaired the immune-modulatory functions of OPC. Our findings suggest that pushing OPC differentiation alone is insufficient to overcome remyelination failure. We suggest that a permissive brain environment is mandatory for remyelination. Furthermore, we highlight the delicate balance between the regenerative and immune functions of the OPC population. Thus, the highly complex mission of creating a pro-regenerative environment depends upon an appropriate immune response controlled in time, space, and intensity. We suggest the need to employ a multi-systematic therapeutic approach, which probably cannot be achieved through a single molecule-based therapy.

Keyword: *Multiple Sclerosis, EAE, OPC, Microglia, Remyelination*

#147 Enhanced liver X receptor signaling reduces brain injury and promotes tissue regeneration following experimental intracerebral hemorrhage: roles of microglia/macrophages

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Even with the development of micro-invasive surgical procedures, inflammation-exacerbated secondary brain injury and limited tissue regeneration impede favorable prognosis after intracerebral hemorrhage (ICH). As a regulator of inflammation and lipid metabolism, liver X receptor (LXR) has the potential to inhibit excessive inflammatory response, alter microglia/macrophage (M/M) phenotype, and assist tissue repair by promoting cholesterol efflux and recycling from phagocytes. Using multimodal magnetic resonance imaging (MRI), quantitative confocal microscopy, real-time PCR, western blot and behavior tests, we observed that the synthetic LXR agonist GW3965 reduced brain injury, and promoted tissue repair and functional recovery following collagenase-induced experimental ICH. In this regard, GW3965 treatment reduced lesion volume and white matter injury and promoted hematoma clearance. Treated mice upregulated LXR downstream genes including ABCA1 and Apolipoprotein E and had reduced density of M/M that apparently shifted from proinflammatory interleukin-1$^+$ to Arginase1$^+$CD206$^+$ regulatory phenotype. Fewer cholesterol crystals, lipid droplets or myelin debris-laden phagocytes were observed in GW3965 mice. Enhanced LXR activation increased the number of Olig2$^+$PDGFRα$^+$ precursors and Olig2$^+$CC1$^+$ mature oligodendrocytes in perihematomal regions, and elevated SOX2$^+$ or nestin$^+$ neural stem cells in lesion and subventricular zone. MRI results supported better lesion recovery by GW3965, and this was corroborated by return to pre-ICH values of functional rotarod activity. The benefits of GW3965 were abrogated by M/M depletion in CX3CR1^{CreER}: Rosa26^{iDTR} mice, suggesting that M/M may mediate these therapeutic effects.

Keyword: *intracerebral hemorrhage, neuroinflammation, liver X receptor, microglia/macrophages, tissue regeneration*

#191 Spheroids on degenerating neurites are eliminated via engulfment, inhibiting degeneration

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Up to 40% of our neurites die as we age, eliminating compensatory circuitry and making us susceptible to neurodegenerative diseases. Furthermore, axonal dysfunction and death precede neuronal death on an order of years in many chronic degeneration contexts[1]. However, there are currently no therapies targeting neurite loss. Axonal spheroids are bubble-like structures that form along all degenerating axons universally[2] and along dendrites in many disease contexts including hypoxia[3], excitotoxicity[4], and tauopathies[5]. Functional implications of spheroids are just beginning to be understood, with recent work finding that spheroids disrupt axon conductance early in Alzheimer's Disease progression[5]. Additionally, previous work from our lab showed that spheroids rupture and release an as-yet unknown prodegenerative factor in vitro[2]. A physiological mechanism for spheroid elimination would therefore confer adaptive protection of the nervous system. We hypothesize that spheroids are eliminated by engulfment, which slows axon degeneration by preventing prodegenerative factor release. To test this hypothesis, we established acute injury models in vivo in zebrafish and in vitro with mouse cell cultures in microfluidic devices. Using

time-lapse, confocal microscopy in these models, we are identifying the phagocytes that engulf spheroids and finding that their engulfment indeed slows axon degeneration. These data identify a new process by which spheroids are eliminated and axon degeneration is regulated. As such, future work will address topics such as whether disruptions in spheroid engulfment contribute to disease pathogenesis, and whether promoting spheroid clearance can rescue disease progression and circuit function.

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Keyword: *Spheroid, Phagocytosis, Axon, Neurite, Degeneration*

#195 Interleukin-1alpha promotes secondary degeneration of oligodendrocytes in mouse spinal cord through activation of astrocytic IL-1R1.

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Spinal cord injury (SCI) often results in loss of integrity in the spinal cord's neuronal network, triggering loss of sensation and voluntary movements below the site of injury. Primary damage is caused by the initial trauma, followed by secondary degeneration mediated by various mechanisms, including inflammation and glial activation. We have discovered that interleukin (IL)-1 alpha, which is constitutively expressed in the healthy CNS and exerts nuclear functions, is released by spinal cord microglia almost immediately after injury. Using knockout mice for IL-1alpha or its receptor, interleukin 1 receptor type 1 (IL-1R1), we observed that both mouse lines exhibited improved locomotion compared to wildtype mice after SCI. We therefore decided to study the effects of IL-1alpha following its injection intra-cisterna magna (i.c.m.), as this

route of delivery is believed to bypass the blood-spinal cord and blood-brain barriers, allowing to investigate the direct effects of the cytokine on CNS-resident cells. Our results show that i.c.m. injection of IL-1alpha in mice induces rapid activation of glial cells throughout the spinal cord, as evidenced by the colocalization of the marker of transcriptional activity Fos with Sox9+ astrocytes and Olig2+ CC1+ oligodendrocytes at 1 and 4 hours post-injection, respectively. Importantly, we observed that IL-1alpha induces the death of mature oligodendrocytes in the mouse spinal cord at 24 hours; a finding that was attributed to IL-1R1 signaling in astrocytes by taking advantage of transgenic mouse lines in which IL-1R1 expression was restored in a cell-specific manner by Cre-mediated recombination. Notably, i.c.m. injection of IL-1alpha to C57BL/6 mice led to a significant increase in the total number of C3-expressing astrocytes on the first day post-injection, an effect that was only replicated by the restoration of IL-1R1 expression specifically in astrocytes and not other CNS cell types. This shows that IL-1alpha is a powerful inducer of astrocytic C3 upregulation, likely associated with a toxic reactive phenotype. Our results suggest that IL-1alpha is an important mediator of secondary degeneration following SCI, and targeting its effect on astrocytes may offer a promising therapeutic strategy.

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Keyword: *Spinal cord injury, Interleukin-1alpha, astrocytes, oligodendrocytes, inflammation*

#204 Microglia-Mediated Neuronal Death can be Suppressed by Selective Cannabinoid Receptor Agonists in Vitro

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Introduction: Neuroinflammation is a hallmark of damage to the brain, and is primarily propagated by microglia, the resident immune cells of the brain[1]. Pro-inflammatory microglia mediate host defense but sustained inflammatory activity can lead to impairment of neurons via accumulation of inflammatory mediators. The

endocannabinoid system modulates immune responses and has been reported to suppress neuroinflammation via actions on microglia which express both cannabinoid type 1 (CB_1) and type 2 (CB_2) receptors[2]. The purpose of this work was to investigate the mechanisms of microglia-mediated neuronal death and determine whether agonists of CB_1 or CB_2 receptors could improve the survival of $STHdh^{Q7/Q7}$ cells which model spiny projection neurons[3].

Methods: Cultured SIM-A9 microglia were stimulated with lipopolysaccharide (LPS) and interferon-gamma (IFNg) to induce a pro-inflammatory phenotype. Release of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-6 were measured using ELISAs and ProteomeProfiler antibody arrays. Conditioned media was collected from microglia and applied to cultured $STHdh^{Q7/Q7}$ neuronal cells for up to 24 h. Neuronal viability was determined using orthogonal assays (CellTiter Glo, CellTiter Blue, Zombie dyes). ACEA (2 nM – 2 µM) was used to activate microglial and neuronal CB_1 receptors. HU-308 (2 nM – 2 µM) was used to activate microglial CB_2 receptors. Neuronal and microglial signaling in response to pro-inflammatory stimuli was monitored using in-cell western assays with antibodies specific for phospho-proteins.

Results: LPS and IFNg induced the release of pro-inflammatory cytokines TNF and IL-6 and reactive oxygen and nitrogen species from microglia. Conditioned media from pro-inflammatory microglia rapidly induced caspase-3 cleavage in $STHdh^{Q7/Q7}$ neuronal cells which culminated in a 57 ± 4% loss in cell viability within 24 h. This was largely mediated by TNF as a 1:100 dilution of neutralizing antibodies reduced neuronal death by 60 ± 3%. When neuronal CB_1 receptors were stimulated, enhanced survival was observed. Furthermore, stimulation of microglial CB_2 receptors indirectly improved neuronal survival through suppression of the pro-inflammatory microglial phenotype. Activation of microglial CB_2 receptors also inhibited LPS-mediated signaling which was the mechanism for the neuroprotective effects.

Conclusions: Microglia in a pro-inflammatory phenotype exhibited the capacity to kill cultured neurons in a TNF-dependent manner. Improved neuronal survival was achieved using three strategies: i) suppression of microglial inflammation by CB_2 activation, ii) neutralization of TNF using polyclonal antibodies, and iii) suppression of neuronal pro-death signaling through CB_1 activation. These data indicate that agonists of CB_1 or CB_2 receptors have potential for therapeutic use in neuroinflammation.

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Keyword: *Microglia, TNF, Cannabinoid, CB2, Apoptosis*

#212 Immune checkpoint molecule Tim-3 regulates microglial function and the development of Alzheimer's disease pathology

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Microglia play a pivotal role in Alzheimer's disease (AD) pathology, though the regulatory mechanism of their function is not fully understood. Havcr2, encoding an immune checkpoint molecule, Tim3, was recently shown as a susceptibility gene for AD. While Tim3 has been shown to play an important role in inducing T cell exhaustion, its role in microglia is unknown. We found that the expression of Tim3 is much higher in microglia than in other cell populations in the brain and other immune cell types. Tim3 gradually increased its expression in microglia during development of the central nervous system. This pattern was similar to the expression change of the genes related to TGF-β signaling, which is critical for microglial homeostasis. We found that the expression of Tim3 was dependent on TGF-β signaling in microglia. Next, we checked the function of Tim3 using microglia-specific Tim3-deficient mice. Tim3-deficient microglia showed increased ability of phagocytosis in vitro and in vivo, with a gene expression profile resembling that of phagocytosing microglia and of MGnD (neurodegenerative microglia phenotype), a specific type of microglia observed in AD models. Importantly, microglia-specific deletion of Tim3 resulted in enhanced clearance of Aβ from the brain in 5XFAD, a mouse model of AD. The phenotype of each plaque was less toxic compared to controls. Cognitive impairment by 5XFAD was not observed in microglia-specific Tim3-deficient background. Molecularly, IP-MS (immunoprecipitatioin-mass spectrometry) screening and confirmatory IP-WB (IP-western blot) analysis showed that Tim3 binds Smad2, a key molecule in TGF-β signaling. The gene expression profile was significantly similar between Tim3-deficient microglia and TGF-β signaling-deficient microglia. Consistently, motif enrichment analysis identified Smad2 as a core transcription factor regulating the transcriptome of Tim3-deficient microglia. Tim3 enhanced phosphorylation of Smad2, which required TGF-β receptor. Detailed analysis showed that the C terminus of Tim3 was necessary for their binding and enhanced phosphorylation of Smad2. Collectively, Tim3 regulates microglial activation through enhancement of TGF-β signaling, thereby inhibiting clearance of Aβ plaques in a preclinical model of AD. Tim3 may be a promising target of new treatment for this intractable disease.

Keyword: *Microglia, Neurodegeneration, Alzheimer's disease, Tim-3, Checkpoint molecule*

#215 Sulforaphane enhances proliferation and maintenance of repair Schwann cells after peripheral nerve injury via anti-inflammatory and cytoprotective Nrf2/HO-1 signaling

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Nuclear erythroid 2–related factor 2 (Nrf2) is commonly activated in response to cellular stresses such as oxidative damage and drives expression of various factors involved in cytoprotection and dampening of inflammatory processes. The activation of the Nrf2/HO-1 signaling pathway has been associated with markedly accelerated peripheral nerve regeneration by clinical, electrophysiological as well as histological measures. However, the exact mechanisms underlying these improvements have not been elucidated so far. To better understand the role of Nrf2 following peripheral nerve injury, we aimed to study the consequences of treatment with the Nrf2 activator sulforaphane (SFN), a naturally occurring isothiocyanate from cruciferous plants, in the murine sciatic nerve crush model.

SFN was administered daily via intraperitoneal injection at a dose of 10 mg/kg, starting immediately after sciatic nerve crush injury was introduced. Animals were sacrificed and sciatic nerves were excised at 7, 14 and 21 days post-crush (dpc) for molecular, immunohistochemical and morphometric analyses. Moreover, functional assessment was performed by grip strength analysis and electrophysiology.

From the end of Wallerian degeneration at 7 dpc, we noted a marked upregulation of the Nrf2/HO-1 signaling pathway under treatment with SFN, which was maintained throughout the entire regeneration phase until 21 dpc. This effect was accompanied by a significant increase in the number of repair Schwann cells as identified by positivity for Sox-2, c-Jun and p75-NTR. In these cells, we also observed elevated proliferation rates identified by Ki67 staining. Concomitantly, apoptotic/autophagic pathways were modulated. These observed changes correlated with a significant clinical improvement in the grip strength test performance, nerve conduction velocity as well as ameliorated histopathological measures at 21 dpc.
Collectively, SFN treatment was associated with an upregulation of cytoprotective pathways, leading to increased numbers of repair Schwann cells that presumably contribute to a permissive environment for successful nerve regeneration. Given the availability of SFN as nutritional supplement, this compound might constitute a novel potentially regenerative therapy with low additional risk of side effects that could be applicable to both mechanical and immune-driven nerve damage and easily combined with existing immune therapies.

#232 Evaluating glial cell response to functional microelectrode implants in vitro

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Insertion of functional microwire implants designed to interface with nervous tissue (e.g., deep brain stimulation, intraspinal microstimulation) elicits an inflammatory response. Central to this response are glia, whose functions include surveillance and defense of the central nervous system. They are also responsible for gliosis, cell death, and glial scar formation that can exacerbate injury and prevent healthy recovery of tissue. The goal of this project is to

develop a systematic approach to evaluating astrocyte and microglia reactivity to implanted neural interface devices. We used 2-dimensional cell cultures as a rapid and reductionist means of assessing glial cell reactivity to chemical and biological stimuli. We placed 75 µm diameter platinum/iridium electrodes with C57BL/6J CX3CR1$^{+/eGFP}$ mouse mixed glial cell cultures in 12-well plates. This enabled the modelling of the glial response to both electrodes and stimulation, and served as a model with increased throughput and reduced ethical footprint which complemented in vivo testing. Time course experiments were performed to document cellular response to biphasic, charge-balanced electrical stimulation applied for 4 h/day over 1, 3, and 7 days. Confocal fluorescence microscopy documented the extent of cellular damage around the electrode interface following experiments, with fluorescence intensity and area coverage quantified for each biomarker (Hoescht, eGFP, GFAP, IL-1beta). Live imaging experiments were also performed to track microglial morphology, movement, and fate in response to modified stimulation parameters. Results from microscopy suggest localized responses at the electrode-culture interface (i.e., increased GFAP, IL-1beta proximal to the electrode), and support published in vivo results. Specifically, it has been previously found that although electrically stimulated animals have had localized increases in GFAP at the electrode interface, such increases were attributed to glial cell response to electrode insertion rather than electrical stimulation itself. Finally, damage to the electrodes themselves following stimulation at different currents was inspected using scanning electron microscopy (SEM), with elemental compositions of each electrode surface quantified using energy-dispersive x-ray spectroscopy (EDS). Using such a platform for testing biocompatibility of different design iterations of wires and stimulation patterns will allow for improvements in implant safety and longevity.

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Keyword: *Neuroinflammation, Microglia, Astrocyte, Electrical stimulation, Microelectrode*

#256 Modulation of Innate Meningeal Infiltrates via Retinoic Acid Receptor Alpha Attenuates Chronic-Progressive EAE and Reduces Tissue Injury

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Background: There is an unmet need for effective therapies in chronic-progressive Multiple sclerosis (CP-MS). Microglia are considered key players in driving neuroinflammation, demyelination, and irreversible neuro-axonal loss in CP-MS. We have identified retinoic acid receptor alpha (RARA) as a powerful modulator of microglia, which can prevent its polarization into a neurotoxic phenotype.

Methods: Effects of Am580, a specific RARA agonist, on primary microglia were examined in vitro. We then continuously delivered Am580 directly to the CNS by using mini-osmotic pumps, in the chronic phase of experimental autoimmune encephalomyelitis (EAE) in Biozzi mice, which mimics closely CP-MS. Clinical course, neuroinflammation, demyelination, and

axonal loss, were evaluated pathologically and by FACS. Single-cell RNA transcriptomics was performed on CD11b+ cells from EAE brains.

Results: The RARA agonist effectively prevented iNOS induction and reactive oxygen species (ROS) production by microglial activators. Continuous intraventricular delivery of Am580 attenuated the clinical severity of chronic-progressive EAE, and reduced axonal injury in the spinal cord. Interestingly, Am580 treatment increased macrophage/microglial and T cell infiltration, and particularly increased the number of meningeal F4/80+ cell infiltrates, with no significant change in parenchymal Iba1+ or TMEM119+ cells. Am580 treatment increased the number of CD11b+ cells isolated from the spinal cords of EAE mice. Single-cell RNA sequencing on isolated CD11b+ cells from both treated and control mice at the chronic phase of EAE indicated that Am580 treatment induces a shift in microglial and CNS-associated macrophages (CAMs) populations towards neuroprotective phenotypes while inhibiting pro-inflammatory pathways. Additionally, Am580 treatment enhanced the presence of F4/80+ sub-populations and concomitantly upregulated antigen-presentation pathways. Moreover, Am580 treatment inhibited apoptosis and regulated cell-death pathways in multiple cell populations.

Conclusions: Meningeal infiltrates are considered a major driver of chronic neuro-inflammation and demyelination in MS. We show here that retinoic acid receptor alpha stimulation of CNS innate immune cells during chronic EAE, increases significantly meningeal infiltration of immune cells with a protective phenotype, resulting in attenuated disease and neuroprotection.

Keyword: *Microglia, MS, EAE, Am580*

#287 Loss of Trem2 signaling disables epigenomic programs associated with Cd11c+ microglia in the demyelinating brain

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Multiple sclerosis (MS) is characterized by demyelinating lesions in the brain white and gray matter. Microglia, the resident macrophages of the brain parenchyma, are involved in active MS lesions by contributing to myelin debris phagocytosis and remyelination. This activity is regulated by surface receptors (e.g., Trem2) which, upon activation, lead to the binding of transcription factors to genomic regulatory elements, promoting transcription of target genes. However, the regulatory landscape of microglia during demyelination and its upstream activators remain under-characterized.

To address this issue, we induced demyelination in mice with the cuprizone diet. After four weeks of treatment, we extracted microglia and used flow cytometry to isolate distinct microglial populations based on Cd11c staining. Various massively parallel sequencing methods (i.e., RNA-seq, ChIP-seq, ATAC-seq) were then employed to detect differential events in the transcriptome and epigenome.

Here we show that the microglial response to demyelination is partly dependent upon transcriptional regulation through Trem2 signaling. A $Cd11c^{+}$ microglia population arises in the cuprizone-treated brain, and RNA-seq data links its transcriptional program to phagolysosome and cholesterol metabolism activity. Furthermore, the landscape of active promoter-distal regions in the $Cd11c^{+}$ population was reprogrammed, with thousands of differentially histone-acetylated sites compared to those of the healthy brain's microglia. Motifs

for certain transcription factors, notably Egr2, Mitf and Mef2, were differentially enriched in those sites. In contrast, Cd11c induction is almost nil in Trem2 KO mice. Early evidence suggests that lack of Trem2 inhibits signals from Egr2, AP-1, C/ebp and Irf transcription factors, but not from Mafb, Mef2 or Smad.

Our results demonstrate that Trem2 is essential to induce the epigenomic programs leading to the phagocytic Cd11c^{+} microglia population in the demyelinating brain.

Keyword: *Microglia, Trem2, Cuprizone, Demyelination, Transcription factors*

#301 Characterization of the role of the transcription factor Mef2a in the regulation of microglial activity in the demyelinating brain

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Background: Most individuals living with multiple sclerosis (MS) eventually develop a progressive form of MS characterized by irreversible worsening of myelin and axon damage. Although the mechanisms underlying the different forms of progressive MS are still unclear, many studies implicate dysregulated microglial inflammatory activity. However, the molecular mechanisms underlying defective microglial activity in demyelinating diseases are not well understood, representing a significant obstacle to our ability to design novel therapies aimed at arresting or reversing the progression of MS. Among the mouse models of myelin damage, there is the Cuprizone (CPZ) model which consists of the ingestion of the CPZ neurotoxin, which is toxic for oligodendrocytes and which leads to a loss of myelin in the brain. Transcriptional studies from the Gosselin lab support, in a preliminary way, the hypothesis that the transcription factor Mef2a, which is highly expressed in microglia, could be a key regulator of microglial inflammatory activity during demyelination.

Methodology: To test this hypothesis, I use mice in which function of Mef2a is suppressed specifically in microglia following tamoxifen administration (Cx3cr1CreERT2/WT::Mef2afl/fl; microglia-Mef2a KO). Objective 1 aims to study the role of Mef2a in healthy adult brain microglia. Objective 2 aims to characterize the contribution of microglial Mef2a activity to the processes of demyelination/re-myelination and phagocytosis/inflammation in brain myelin pathologies. For this, microglia-Mef2a KO mice will be compared to control mice (Cx3cr1CreERT2/WT::Mef2aWT/WT) during cerebral demyelination using CPZ model.

Results: Data indicate that the absence of Mef2a impairs microglia from differentiating into CD11c^{High}microglia and that this significantly interferes with the myelin repair process. The repair deficit is accompanied by an exaggerated microglial accumulation, as revealed by a more intense labeling of the CD68 phagocyte marker in Mef2a-microglia KO mice than in WT controls.
Conclusion and perspectives: These data suggest that the absence of Mef2a in microglia prevents remyelination and leads to an exacerbation of inflammation in the demyelinating brain. I am currently carrying out epigenomic analyzes to understand the underlying mechanisms. Together, my data seem to support the hypothesis that Mef2a mediates the proper microglial activity necessary to limit demyelination and/or promote myelin repair.

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Keyword: *Neuroinflammation, Multiple sclerosis, Microglia, Demyelination, Transcriptional regulation*

#306 Investigating neurotoxicity of iron deposited into the mouse spinal cord and in vitro

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Substantial evidence shows the accumulation of iron in the central nervous system (CNS) in many neurodegenerative diseases such as Multiple Sclerosis, Alzheimer's disease, and Parkinson's disease. Iron homeostasis must be tightly regulated, as free and poorly liganded iron can produce free radicals and lead to ferroptosis. However, the extent of iron toxicity in various CNS cells is poorly understood. Here, we evaluate whether neurons and microglia have a differential vulnerability to iron and examine the potential mechanisms of injury. We microinjected ferrous iron into the ventrolateral white matter of the mouse spinal cord and harvested tissues at different time points. Surprisingly, while the white matter appears relatively intact, the neighbouring grey matter has extensive loss of $NeuN^+$ neurons by one-day post injury (dpi) and accumulation of $CD68^+$ microglia and macrophages by three dpi. There is also a prominent representation of microglia/macrophages with transferrin receptor, an iron importer, in the grey matter, suggesting that during iron overload, $CD16/32^+$ microglia/macrophages may enhance iron uptake to remove the excess threat from the extracellular environment to minimize oxidative damage. Additionally, the absence of glutathione peroxidase 4 enzyme in these iron-induced lesions implies that ferroptosis is occurring. In tissue culture, neurons are highly susceptible to iron-induced death. Impressively, this neuronal killing by iron is exacerbated by microglia. Continuing experiments seek to uncover new factors and mechanisms involved in iron toxicity in grey matter, especially in neurons. Such insights should shed light on how iron contributes to the pathogenesis of MS and enhance our understanding of the role of immune cells in protecting or injuring the CNS against iron.

#313 Region-specific astrocyte alterations underlie chronic stress response in male mice

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Major depressive disorder (MDD) is a severe neuropsychiatric illness that will affect 20% of the population throughout lifetime and is a major cause of disabilities according to the WHO. Unfortunately, 30-50% of individuals with MDD respond poorly to currently available treatment suggesting that causal mechanisms, such as increased circulating inflammation and neurovascular dysfunction, remain untreated. Alterations of the blood-brain barrier (BBB), formed by endothelial cells, pericytes and astrocytes, are observed in individuals with MDD and after exposure to chronic social defeat stress (CSDS), a mouse model of depression. Chronic stress is the main environmental risk factor to develop MDD and it is associated with increased

circulating levels of inflammatory cytokines namely interleukin-1beta, interleukin-6 and tumor necrosis factor-alpha. Stress-induced BBB leakiness allows the passage of inflammatory mediators from the blood into the brain possibly contributing to neuronal dysfunction and depressive behaviors. Astrocytic morphological changes such as reduced end-feet coverage of blood vessels, occur in the MDD brain and are associated with inflammation and impaired function of these glial cells necessary for proper brain homeostasis. However, possible contribution to MDD pathogenesis and maladaptive stress responses remains to be determined. Male mice were subjected to 10-day CSDS producing two subpopulations: stress-susceptible (SS) animals characterized by depression-like behaviors and resilient (RES) mice behaving like unstressed controls. CSDS induces BBB hyperpermeability mostly in the nucleus accumbens (NAc), a hub for mood regulation, reward processing, and stress responses. Reduced gene expression of connexin gap-junctions, linking neuronal and vascular activity, was observed in the NAc of SS, but not RES, male mice. Conversely, increased expression of growth factors and inflammatory markers was measured in the prefrontal cortex of RES animals, supporting compensatory mechanisms in this brain area important for decision-making and social behaviors possibly to counteract the deleterious effect of stress-induced inflammation. Functional measurements are ongoing to better define the role of astrocytes in the development of depression-like vs proper stress-coping behaviors. Altogether, these results suggest that astrocytes could actively contribute to susceptibility vs resilience to chronic stress exposure, and possibly MDD, in a brain region-specific manner.

#336 Spatial rearrangement of PDGFR-beta cells at the interface of glial scar following brain injury

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Tissue scarring is a fundamental aspect of the central nervous system (CNS) repair after injury. PDGFR-beta cells are a group of perivascular cells (comprising pericytes) that actively respond to injury and reorganize at the interface of the glial scar. Here, we use point pattern analysis (PPA) and topological data analysis (TDA) to dissect the topological rearrangement of PDGFR-beta cells and their contribution to tissue scarring after cerebral ischemia. We subjected transgenic Rosa PDGFR-beta P2ACreER mice to 30 min middle cerebral artery occlusion (MCAO) and performed immunohistochemistry for glial scar-forming cells to analyze their covariance with Td-Tomato+ PDGFR-beta cells. Our preliminary results indicate that reactive PDGFR-beta cells dissociate from the vasculature and massively accumulate in the injury core from the first week after cortico-striatal ischemia. Interestingly, these cells acquire a uniform morphology and are later segregated by GFAP+ scar-forming astrocytes to the outermost borders of the injured cortex. This implies that these PDGFR-beta cells are completely deprived of life support. Furthermore, PDGFR-beta cells remain associated with the vasculature only in striatal ischemic lesions, suggesting an alternative reactive profile depending on the type of injury. Moreover, PDGFR-beta cells in perilesional regions undergo marked morphological transformations that can be analyzed quantitatively.

Keyword: *Glial scar, PDGFR-beta, Cerebral ischemia, Neurovascular reactivity, Pericytes*

#347 Pleiotrophin as a modulator of neurite outgrowth, neuroinflammation and OPC differentiation in the presence of CSPGs

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After CNS injury such as ischemic stroke, chondroitin sulphate proteoglycans (CSPGs) are produced by activated glial cells in the extracellular matrix surrounding the injury. CSPGs are growth inhibitory, reducing axonal sprouting growth and migration of OPCs and thereby impairing recovery. Pleiotrophin (PTN), a growth factor and a cytokine, is upregulated in the central nervous system (CNS) during development and after injury. However, the effect of CSPGs and PTN on different classes of glial cells is not well described. Here, we investigated the effect of PTN and PTN signaling on primary neuronal and glial cultures. First, neurons were plated on growth inhibitory CSPG matrices or growth permissive laminin matrices and treated with varying concentrations of PTN. Notably, PTN induced growth even on the inhibitory CSPG matrix, and this growth was dependent on activation of ALK receptor. Next, OPCs, microglia, and astrocytes were isolated from mixed mouse glia cultures and plated on growth inhibitory CSPG matrices or growth permissive laminin matrices and treated with varying concentrations of PTN. PTN promoted the differentiation of OPCs to mature oligodendrocytes on CSPG matrices. Microglia plated on CSPG matrices induced the breakdown of CSPGs by increased release of MMP 9 and MMP 2. Moreover, treatment with PTN in the presence of CSPGs reduced the release of IL6, MCP1, IL 10 and TNF from microglia and with increased microglial phagocytosis and proliferation.. Combined, these data suggest that PTN signaling modulates axonal growth, remyelination process and promotes anti-inflammatory response even in inhibitory environments, thus may have potential as a pro-plasticity therapy following CNS injury.

Keyword: *Neurons, Microglia, OPCs, CSPGs, PTN*

#353 Biophysical functional characterization of missense variants of voltage-gated calcium channel CaV2.1 (CACNA1A)

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CACNA1A encodes the low-voltage-activated $Ca_V2.1$ P/Q-type calcium channel that is highly expressed in Purkinje neurons of the cerebellum, as well as throughout the cortex and other regions of the brain. Additionally, CACNA1A has been shown to be expressed in Oligodendrocyte precursor cells (OPCs) and be implicated in the maturation stage into oligodendrocytes. Cav2.1 channels play an important role in major calcium influx mechanisms that underlie neuronal excitability and presynaptic neurotransmitter release. Altered functionality of CACNA1A has been associated with various pathologies ranging from Spinocerebellar Ataxia S6 (SCA6) to Multiple Sclerosis (MS), however, the molecular phenotypes of CACNA1A underlying the pathophysiology of these disorders are still elusive. Here, we analyzed an allelic series of 40+

de novo missense changes of CACNA1A identified in a large cohort of 31,058 parent–offspring trios of individuals with developmental disorders. In addition, we included 6 novel de novo or likely de novo variants of CACNA1A from patients at Boston Children's and Children's Hospital of Philadelphia (CHOP) in our analyses to characterize the functional properties of CACNA1A variants implicated in CACNA1A disorders. We performed functional evaluation of CACNA1A missense variants using automated patch-clamp (SyncroPatch384) and compared the functional properties among of Cav2.1 channels encoded by de novo variants with those of channels harboring coding changes identified in normal control subjects in the Genome Aggregation Database (gnomAD). We analyzed five biophysical properties, including the whole cell current density, the voltage-dependent activation/inactivation and the kinetics of inactivation/deactivation. Majority missense variants encode channels with significantly reduced current densities compared those identified in gnomAD. Interestingly, several missense variants from neurodevelopmental cohorts showed leftward shift in voltage-dependent activation, while majority of missense variants exhibited rightward shift in voltage-dependent inactivation. Having used HEK293 overexpression to get a clean readout of Cav2.1 channels, our data can be used to partially inform calcium dependent aspects of myelination in MS. Taken together, the results of our functional analysis of an allelic series of CACNA1A variants from cohort and control groups may provide important insights on the role of CACNA1A dysfunction in disorders and disease.

#360 Mapping the Functional Interactome in MS Lesions Relevant to Remyelination Failure

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Background

Despite substantial scientific interest and several clinical trials, remyelination remains a critical unmet therapeutic need in multiple sclerosis (MS). While single nucleus RNA-Seq (snRNA-Seq) studies of MS lesion tissue have greatly expanded our ability to define cellular subpopulations, additional biological insight can be obtained by adding spatial information. We integrated snRNA-Seq data with highly multiplexed imaging of MS lesions to determine spatial cellular patterns, including spatial interactions and receptor-ligand (R-L) interactions between neighboring cell types. With this, we are aiming to identify key cellular interactions that prevent remyelination and can be targeted therapeutically.

Objectives

To identify inhibitory pathways that lead to remyelination failure and dormant pro-myelinating pathways that may respond to therapeutic stimulation.

Methods

We performed highly multiplexed imaging (iterative indirect immunofluorescent imaging [4i]) on MS lesion tissue using an antibody panel designed to reflect snRNA-Seq-derived cellular populations. We employed a computational analysis pipeline (Image J, Squidpy, CellPhoneDB) to determine the localization of different cellular subpopulations within the lesion environment, their spatial interactions with other cell populations and their functional R-L interactions with neighboring cells.

Results and Conclusions

With this approach, we spatially resolved snRNA-Seq-defined cell clusters. We were further able to phenotypically refine these clusters based on

their localization within the lesion, and their cellular interactions and protein expression patterns. Notably, we found that cellular stress levels increased in pre-myelinating oligodendrocytes with increasing proximity to the lesion center. This was assoicated with changes in spatially interacting cell types and R-L interactions, ranging from homeostatic in normal appearing white matter to increasingly reactive astroglial subpopulations in the lesion rim and lesion center. We are currently verifying the key interactions that induce cellular stress and testing their ability to inhibit myelination.

In summary, combining snRNA-Seq data with highly multiplexed imaging is a powerful approach to phenotypically annotating cell populations, identifying key cellular interactions in MS lesions and ultimately designing remyelinating therapies.

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Keyword: *multiple sclerosis, remyelination, 4i, sNuc-Seq, functional interactome*

#374 S1P receptor modulators support partial protection from astrocyte-induced neurodegeneration and demyelination

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Astrocytes response to neuroinflammation may be beneficial or detrimental for tissue repair, depending on the activation of distinct intracellular signalling pathways. We demonstrated previously that two approved drugs for multiple sclerosis targeting S1P receptors (S1PR), fingolimod and siponimod, may hamper cytokine signalling in astrocytes and attained neuroprotection indirectly. On the other hand, activation of the neurotrophin receptor TrkB in astrocytes contributes to nitric oxide-induced neurodegeneration and copper-mediated demyelination.

Here we address whether S1PR modulators may interfere with astrocyte TrkB signalling in neuroinflammation.

We checked the involvement of S1PR signaling in the modulation of TrkB expression and observed

that exposure of primary mouse astrocytes to the drugs inhibited IL1-induced TrkB up-regulation. Similarly, therapeutic administration of S1PR modulators to mice with experimental autoimmune encephalomyelitis (EAE) reduced astrogliosis and TrkB protein in white matter spinal cord.

While TrkB signaling induced calcium flux and NFkB translocation in astrocytes exposed to BDNF, these processes were inhibited by S1PR modulators, indicating that the S1PR signaling may sustain initial steps of TrkB mediated astrocyte activation. In vitro studies on primary spinal neurons showed that astrocyte conditioned media (ACM) from BDNF-treated cells induced neurodegeneration, while ACM generated in the presence of drugs did not affect neuronal survival, demonstrating that targeting S1PR signalling in astrocytes may rescue neurons from toxicity due to astrocyte TrkB activation.

We then investigated whether S1P signaling could interfere with astrocyte-driven copper trafficking. S1PR modulators impaired the induction of the copper transporter CTR1 on glial cells during EAE and in primary cells exposed to IL1 or BDNF. In vitro assays on rodent oligodendrocytes (OL) showed that ACM from copper-exposed astrocytes triggered OLs death even when media were generated in the presence of fingolimod or siponimod. Although in vivo drug administration to EAE mice ameliorated clinical symptoms, inflammation and neurodegeneration, myelin content was similar in the spinal cord of vehicle- and drug-treated animals, suggesting that S1PR modulators do not mitigate astrocyte-dependent copper-induced demyelination.

Our observations indicate that S1P receptor modulators may partially interfere with astrocyte function during neuroinflammation, thus supporting neuroprotection but not myelin rescue.

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#390 Regulation of microglial TNF production by noncoding RNAs in multiple sclerosis lesions

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Multiple sclerosis (MS) is characterized by inflammatory lesions with infiltration of autoreactive T cells leading to demyelination and axonal degeneration, as well as activation of microglia and upregulation of the microglial production of the cytokine, tumor necrosis factor (TNF). TNF has pleiotrophic functions in MS, however avoiding high local levels of soluble TNF would probably be protective. We have previously shown that infiltration of interferon-ɣ (IFN-ɣ)-expressing myelin-specific T cells into zones of axonal degeneration, exacerbates the axonal-lesion-induced microglial activation (1,2,3), leading to increased expression of TNF in microglia (unpublished data). This study tests the hypothesis that the microglial production of TNF is regulated, in MS-like lesions in mice as well as in T cell-infiltrated MS lesions, by IFN-ɣ through specific regulatory, noncoding RNAs (ncRNAs). Through RNA sequencing studies on RNA samples from IFN-ɣ- and vehicle-stimulated primary, murine microglia, we have selected six TNF-related ncRNAs including two potential TNF

mRNA-targeting microRNAs to investigate further. Ongoing studies use in situ hybridization (ISH) and immunohistochemical techniques to detect the ncRNAs and different microglial and T cell markers, and TNF, respectively, in our murine model for MS and in autopsies from deceased MS patients with or without T cell-infiltrated lesions. Next, we will combine the use of LNA-probes with immunofluorescence to localize the ncRNAs to TNF^{high}- and TNF^{low}-expressing microglia/macrophages. The clinical perspective is to identify new TNF-modulating therapies to be used in patients with MS.

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Keyword: *T cells, Interferon gamma, Microglia, TNF, non-coding RNA*

Microglia in neural development, remodelling, and protection

#111 Characterization of microglial states including DM during postnatal development, in health and MIA induced with Poly I:C

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Microglia are needed throughout life, but a particular area of intrigue are the critical roles they play in development making them an area of interest when it comes to neurodevelopmental disorders, particularly those arising from exposure to maternal immune activation (MIA). MIA is any inflammatory response in utero that can alter fetal neurodevelopment, placing exposed offspring at increased risk of neurodevelopmental disorders. MIA can be triggered by a variety of environmental factors such as viral infection (e.g., COVID- 19), psychological stress and unbalanced diets. A microglial subtype of specific interest for this project is dark microglia (DM) which are strikingly different from other microglia when viewed with electron microscopy and make extensive interactions with the vasculature and synapses, which suggests that DM play a role in vascular and synaptic remodeling. DM are abundant during normal CNS development but rare in healthy adults, and they increase in number with environmental challenges such as viral infections (which can be modelled in rodents with polyinosinic:polycytidylic acid or poly I:C). We will be looking at the hippocampus, where DM are very abundant, as it contributes to many cognitive functions involved in neurodevelopmental disorders. To test this, C57BL/6J female mice were injected with Poly I:C 9.5 days into pregnancy to induce MIA. We perfused MIA offspring mice at postnatal day 10, 15 and 20 (timepoints corresponding to major periods of vascular and synaptic maturation in the hippocampus). We examined both males and females as sex-dependent mechanisms are a key effector of the sex differences seen in some neurodevelopmental disorders. The brains were

cut on a vibratome and stained with immunohistochemistry for markers that allow to visualize DM (e.g., TREM2 and Clec7a) versus other microglia (e.g., TMEM119 and IBA1). The sections were examined across postnatal development, in males and females, during health and MIA (n=10–12 mice per sex/condition). Epifluorescence microscopy allows us to determine the density and distribution of DM versus other microglia, while confocal microscopy using z-stacks which provides increased spatial resolution will allow to study the morphology–linked to physiological functions such as surveillance–of DM versus other microglia. To gain insights into possible changes in phagocytosis, I will also use the marker of phagolysosomal activity CD68. We hope that this work will provide insight into how MIA can lead to changes in the brain and behaviour through altering microglia.

Keyword: *Micoglia, Development, Maternal Immune Activation, Dark microglia*

#162 Impact of a change in microbiota on microglia during brain development

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Microglia are brain cells involved in different functions, such as immune response or brain development. Through their phagocytic activity, microglia can eliminate cells or prune synapses, thereby controlling the formation of neuronal networks. A potentially important influence in this process is the intestinal microbiota, which has been shown to modulate the microglia. To learn more on the impact of the gut microbiota on the phagocytic function of microglia during CNS development, we are establishing an optogenetic larval zebrafish model.

Keyword: *Microglia, Brain development, Microbiota, 2Photons imaging*

#163 Differential microglial response to sleep deprivation depending on the hippocampal region and the paradigm used in adult male mice

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In our society focused on efficiency and performance, the lack of sleep has now become the new normal. However, sleep loss can lead to many detrimental consequences, including cognitive impairments. In the brain, the hippocampus is one of the regions that is most vulnerable to sleep deprivation, often leading to neuronal connectivity changes and a reduction of synaptic density. Strikingly, sleep deprivation leads to subregion-specific changes affecting the Cornu Ammonis (CA)1, but not CA3 at the structural plasticity level. Microglia, the resident immune cells of the brain, are important contributors to synaptic plasticity, and their functions are affected by sleep deprivation. As microglia are a highly heterogeneous population and their functions can differ among brain regions, we aim to determine if microglia could

be involved in the region-dependent synaptic deficits observed after 2 different paradigms of sleep deprivation. To do so, we exposed male mice to 6 hours of sleep deprivation, which is representative of what can be experienced in everyday life, through either gentle handling (GH) or exposition to novel objects (NO). Using double immunofluorescence staining for ionized calcium-binding adaptor molecule 1 (IBA1), a marker of microglia and macrophages, and for transmembrane protein 119, a marker more specific to microglia, we confirmed there was only marginal infiltration by peripheral myeloid cells in all conditions. Then, using immunostaining against IBA1, we observed a decreased microglial density in the NO paradigm compared to control and GH paradigm, in the CA1 stratum radiatum, but not the CA3 stratum radiatum. This was accompanied in the CA1 stratum radiatum by an increased nearest neighbour distance in both GH and NO paradigms compared to controls. We also characterized microglial phagocytic activity using a double immunofluorescence against IBA1 and cluster of differentiation 68, a phagolysosomal activity marker. We found an increased number of CD68 puncta co-labelled with IBA1 cells in the NO paradigm compared to both controls and GH in the CA1 stratum radiatum, but no effect was observed in the CA3 stratum radiatum. To further characterize microglial functions and interactions with synapses, we will evaluate possible changes in their morphology, as well as their interaction with pre-synaptic elements. We will further characterize their ultrastructure using scanning electron microscopy to provide a better understanding of their interactions with synapses. These first results indicate that microglia behave differently depending on the sleep deprivation paradigm used and the hippocampal region. Elucidating the role of microglia in the synaptic deficits resulting from sleep deprivation may ultimately contribute to developing novel therapeutic strategies aimed to combat the negative impact of sleep deprivation on cognitive function and brain function as a whole.

Keyword: *Microglia, Sleep deprivation*

#364 Deletion of Pten in microglia promotes myelin repair in the cuprizone model of demyelination

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Background: Increasing evidence suggests that inhibiting functions of the protein phosphatase and tensin homolog (Pten) in the adult brain can promote repair following brain injuries [1, 2]. As a phosphatase, Pten is a potent negative regulator of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, which is a critical modulator of cell survival and metabolism, and protein translation [3, 4]. Microglia, the parenchymal resident macrophages of the brain, robustly express Pten. However, the role of Pten in regulating microglial cell biology is not well defined. Furthermore, whether suppressing Pten functions in microglia can enhance their pro-remyelinating activity in the demyelinating brain has yet to be determined.

Methodology: To address these knowledge gaps, I conducted a series of experiments to characterize the role of Pten in microglia in vivo in demyelinating disorders. For this, I generated mice in which Pten activity is suppressed specifically in microglia following tamoxifen administration (Cx3cr1CreERT2/WT::Ptenfl/fl;

microglia-Pten KO); Cx3cr1CreERT2/WT::PtenWT/WT mice were used as controls. To study myelin repair, I placed mice under the cuprizone diet for 5 weeks and then returned them to a regular diet for one additional week. Mice were then sacrificed for analyses (imaging and flow cytometry).

Results: To date, my results show that the absence of Pten enhances signal intensity of modified Gallyas staining, which labels myelinated axons, in the corpus callosum one week after the termination of the cuprizone diet. Furthermore, while microglia/macrophage accumulation over the same region is similar in controls and microglia-Pten KO mice, flow cytometry analyses revealed that the absence of Pten significantly augments the proportion of CD11cHigh microglia. Flow cytometry analyses also did not reveal the effect of Pten in promoting microglial proliferation, as assessed by Ki67 expression.

Conclusion and perspectives: Overall, my results indicate that inhibiting Pten functions promotes remyelination in the brain and that this may occur as a consequence of enhanced CD11c high microglial activity. Current experiments are investigating the underlying signaling, epigenomic, and transcriptional mechanisms possibly implicated.

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#368 Novel Cell Population a Key to Brain Rejuvenation

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Aging is an inevitable process that results in biological, anatomical, and functional declines, as well as cognitive impairments. Although it is not considered a disease by itself, an aging brain is more vulnerable to immune stimuli in comparison to a young brain. As a result, aged cells in the central nervous system (CNS) show a diminished capacity to effectively carry out their functions, leading to a decline in their ability to repair damage within the CNS. This, in turn, gives rise to many immunological and neuronal responses, which are associated with impairments in synaptic plasticity, neurogenesis, and cognitive abilities, ultimately leading to a decline in brain function. Given these changes, there is growing interest in targeting these detrimental processes as a potential way for discovering effective treatments for neurodegenerative diseases associated with aging. In our research, we have identified a unique subset of microglia that play a critical role

in neurogenesis within the developing brain. Intriguingly, our recent findings demonstrate that transplantation of these cells to the cerebrospinal fluid of aged mice, yields remarkable improvements in cognitive abilities among these animals including enhanced recognition memory in the Novel Object Recognition task and improved short- and long-term memory in the Barnes Maze task, which was comparable to the outcomes observed in untreated young mice. Building upon this outcome, we aim to investigate the underlying mechanism behind this therapeutic effect. This comprehensive project holds the potential in paving the way for the development of innovative therapies targeting age-related neurological conditions as well as other neurodegenerative diseases.

Keyword: *microglia, aging, memory, neurodegeneration, cognition*

#369 The role of microglial IGF-1 in neurodevelopment

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Insulin- like growth factor 1 (IGF-1) is a peptide hormone expressed in different tissues of the human body, its mechanism of action depends on the place of the secretion. Its presence is essential for proper development and growth, complete knockout of IGF-1 in mice is lethal, causing microcephaly, growth retardation and defects of organs. Our group has identified microglia as the main source of IGF-1 in the developing brain and its critical role in primary myelination. In this project we aimed to investigate impact of microglial IGF-1 for development and functions of the brain. We have generated microglia specific inducible conditional knockout of IGF-1 and induced its depletion in new-born pups. Behavioural tests including marble burying, open field and novel object were performed on mice 13 weeks after IGF-1 depletion. The deficiency of microglial IGF-1 lead to growth retardation manifested by significantly lower body and brain weight. Moreover, we observed behaviour abnormalities presented as excessive grooming, increased anxiety and neophobia. Immunohistochemistry and qPCR analysis demonstrate changes in microglia numbers and activation. All in all, these results show importance of microglia-derived IGF-1 for brain development and function and open new perspectives for investigation of the role of microglial-IGF1 in neurological diseases.

Keyword: *IGF-1, neurodevelopment, microglia, behaviour*

#382 Microglia- neuroprotective cells of the CNS.

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Microglia are central nervous system (CNS)-resident immune cells. They are implicated in neuroinflammatory and neurodegenerative diseases including multiple sclerosis. We have shown that numbers of microglia expressing CD11c, normally almost undetectable in adult CNS, significantly increase in experimental autoimmune encephalomyelitis (EAE). These are effective antigen-presenting cells, but poor inducers of pathogenic T-cell responses. Interestingly, $CD11c^+$ microglia express high levels of neuroprotective insulin-like growth factor 1 (IGF1), suggesting their neuroprotective rather than pro-inflammatory role. We have recently shown that $CD11c^+$ microglia

cells predominate in primary myelinating areas of the developing brain and express genes for neuronal and glial survival, migration and differentiation, and they control primary myelination via IGF1 production.

Here we show that upon adoptive transfer into the cerebrospinal fluid of adult mice with symptomatic EAE, neonatal microglia migrate to the inflammatory lesions in the spinal cord. This intervention suppressed disease symptoms and reduced leukocyte infiltration and demyelination. Although unfractionated neonatal microglia suppressed the disease, the CD11c+ microglial subset was most effective. We therefore identify a unique phenotype of neonatal microglia that have re-myelinating and anti-inflammatory potential. Understanding mechanism for these protective effects will enable therapy for neuroinflammatory diseases.

Neuroimmunology general I

#7 Neutrophil extracellular traps trigger B cell death in intestinal Peyer's patches after stroke

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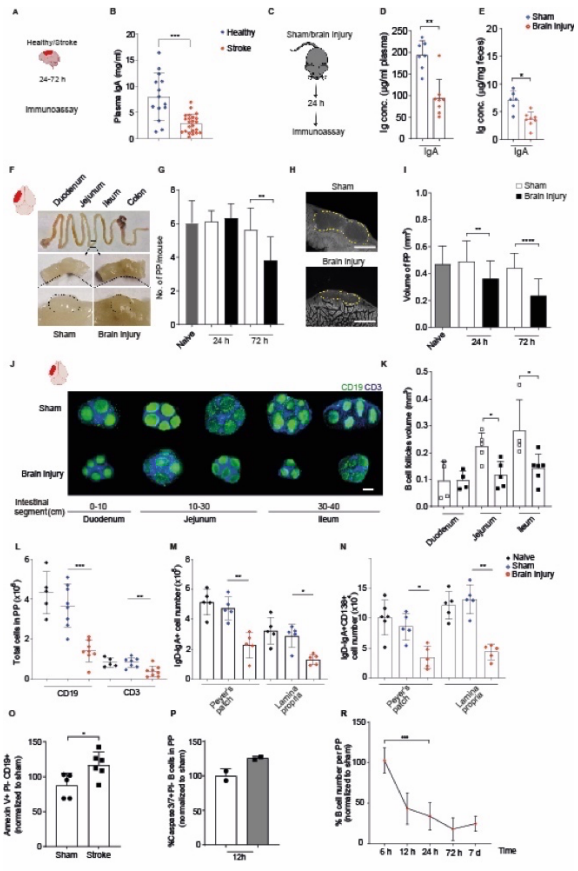
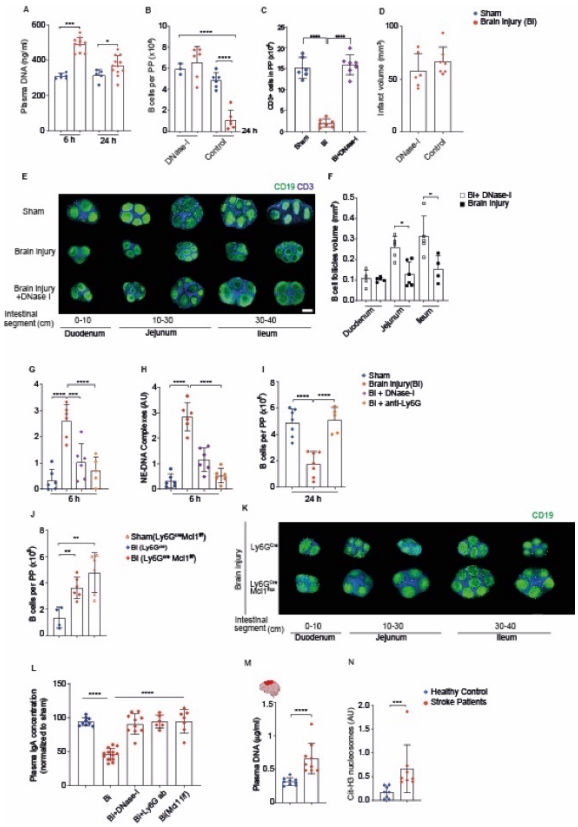
Stroke is the second most frequent cause of death and the third most prevalent cause of human disability in the world. Even though there have been improvements in the treatments of stroke and stroke related complications, post-stroke infections are still one of the major reasons of mortality and morbidity worldwide (1). In order to investigate the pathophysiology of this condition, we collected blood samples from the patients in stroke unit during the first week of stroke onset and observed reduced levels of plasma Immunoglobulin A (IgA).

Commonly used animal model of stroke (transient middle cerebral artery occlusion, tMCAO) also showed decreased IgA levels as the stroke patients. We observed immune cell loss in several immune compartments, such as blood, spleen and interestingly Peyer's patches (PP). As PP are important hubs for B cell development in the gut associated lymphoid tissue (GALT) (2), but how sterile tissue injury leads to cell loss in PP has not been explored, we investigated the immune cell loss in PP mechanistically, starting from 6 hours until 7 days post stroke. We observed rapid and macroscopically evident shrinkage of PP after stroke. Light-sheet fluorescence microscopy and flow cytometry revealed a strong reduction in the number of PP-resident B cells. Mechanistically, tissue injury triggered the activation of

neutrophils that released neutrophil extracellular traps (NETs) decorated with citrullinated histone-H3 (cit-H3), that cause B cell apoptosis. Antibody-mediated or genetically induced neutrophil-loss, NETs-degradation or blockade of their generation completely reversed B cell loss and preserved the tissue architecture of PP.

We also found cit-H3 and neutrophil elastase decorated DNA, components of NETs, in human post-stroke plasma. Hence, we propose that targeting NET-generation or -function counteracts post-injury B cell death in PP and thereby maintains immune homeostasis at mucosal barriers. Targeting NETs after stroke might be a new therapeutic intervention in patients with stroke to decrease mortality and morbidity after stroke.


A
Healthy/Stroke
24-72 h
Immunoassay
B
Plasma IgA (mg/ml)
Healthy
Stroke
C
Sham/brain injury
24 h
Immunoassay
D
Ig conc. (µg/ml plasma)
IgA
E
Ig conc. (µg/mg feces)
IgA
Sham
Brain injury
F
Duodenum
Jejunum
Ileum
Colon
Sham
Brain injury
G
No. of PP/mouse
Naive
24 h
72 h
H
Sham
Brain injury
I
Volume of PP (mm³)
Sham
Brain injury
Naive
24 h
72 h
J
CD19 CD3
Sham
Brain injury
Intestinal segment(cm)
0-10
10-30
30-40
Duodenum
Jejunum
Ileum
K
B cell follicles volume (mm³)
Duodenum
Jejunum
Ileum
L
Total cells in PP (x10⁶)
CD19
CD3
M
IgD+IgA+ cell number (x10³)
Peyer's patch
Lamina propria
N
IgD-IgA+CD138+ cell number (x10³)
Peyer's patch
Lamina propria
Naive
Sham
Brain injury
O
Annexin V+ PI- CD19+ (normalized to sham)
Sham
Stroke
P
%Caspase3/7+PI- B cells in PP (normalized to sham)
12h
R
% B cell number per PP (normalized to sham)
6 h
12 h
24 h
72 h
7 d
Time



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Keyword: *stroke, Peyer's patch, immunosuppression, neutrophil extracellular traps, B cells*

#28 Modulatory Effect of Muscarinic Acetylcholine Receptors on the Human Memory CD3+CD4+CD45RA-CD45RO+ T Cells

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Introduction:

Memory T helper (Th) cells are a crucial part of the adaptive immune system, generated following a primary immunogenic challenge and are capable of producing functional cytokines in response to subsequent pathogen exposure. T cells are influenced by several neuroimmunological factors. Acetylcholine (ACh) is a neurotransmitter that is produced by parasympathetic nervous system and influences the immune system via nicotinic and muscarinic acetylcholine receptors (mAchRs). Five mAChR subtypes (M1-M5) have been detected in human T cells, but their function is not completely understood. This study aims to evaluate the effect of M3 mAchRs on the cytokine production and proliferation of the ex vivo isolated memory Th cells.

Method:

Memory Th cells were isolated from the peripheral blood of healthy participants by immunomagnetic sorting. The average purity of CD3+CD4+CD45RA-CD45RO+ memory Th cells was 98.9% ± 0.64% as determined by flow cytometry. During activation with anti-CD3/anti-CD28/anti-CD2 conjugated antibody (immunocult), memory Th cells were exposed to 100 µM oxotremorine-M, a muscarinic acetylcholine receptor agonist, and/or 10µM atropine and 4-DAMP (M1-M5 mAchRs and M3

mAchRs antagonists, respectively) for five days of incubation. ELISA, western blot, flow cytometry, and RT-qPCR techniques were employed to analyze the production of IL-4, IL-17A, and IFN-γ, activation of nuclear factor kappaB (NF-κB). Cell proliferation was assessed with CFDASE dye and flow cytometry.

Results:
Stimulation of mAchRs via oxotremorine-M led to a significant increase in the levels of proinflammatory cytokines IL-17A and IFN-γ, while decreasing the levels of the anti-inflammatory cytokine IL-4 ($p<0.001$). These effects of oxotremorine-M were blocked by atropine and 4-DAMP, indicating the specificity of the agonist's effects. These data demonstrate that the generation of proinflammatory cytokines is mediated by M3 mAchRs via inducing the activation of NF-κB ($p<0.05$). Oxotremorine-M did not change the proliferation of memory Th cells.

Conclusion:
Our study reveals that M3 mAchRs play a critical role in regulating the activation of memory Th cells towards a proinflammatory phenotype, potentially via activation of the NF-κB signaling pathway. This novel insight into the cholinergic control of immune function highlights the importance of understanding the complex interplay between the nervous and immune systems in regulating immune responses. These findings could pave the way for the development of novel vaccines and therapeutic strategies against various pathogens by targeting the cholinergic system in memory Th cells.

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Keyword: *Acetylcholine, Cholinergic System, NF-κB, Oxotremorine-M, Memory Th Cells*

#37 Clinical, Biological, Imaging and Genetic Repository (C-BIGR); An Integrated Approach to Biobanking in the Context of Open Science

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The ability for physicians to treat patients with neurological disease is often limited by the incomplete understanding of how and why these diseases arise and why they behave the way, they do. To improve our knowledge, the Montreal Neurological Institute's Open Science Clinical Biological Imaging and Genetic Repository (C-BIGR) will help to better assess that biology and its impact under treatments. The main objective is to collect biological material as well as clinical,

imaging and genetic information from patients and controls in order to enable innovative research projects that will advance our understanding of neurological diseases and human health under the Open Science principles. To protect patient identity, a global unique identifier (GUID) identifies all materials received. Open, registered and controlled access data are available to researchers and industries via an online-encrypted database to avoid any re-identification of the data. Furthermore, a committee examines each research proposal for its scientific and ethics value to ensure the donor's anonymity. C-BIG standard operating protocols (SOPs) govern the preparation and the storage of biological materials. Since 2016, material and data from more than 3,000 donors are available in the repository. More than 45 collaborations with researchers and industries have used our biological material and returned data and IPSC cell lines (more than 200 lines) to CBIG. The long-term goal is to develop a comprehensive collection of data and samples on a diverse array of neurological conditions, hoping to contribute to global translational neuroscience research by increasing collaboration with researchers and industries.

Keyword: *Biobank, Repository, Open Science*

#47 Investigating Metabolic Modulation of Microglia during Multiple Sclerosis Pathogenesis: Role for EMMPRIN/MCT-4 Axis

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Multiple sclerosis (MS) is a demyelinating and degenerating condition of the central nervous system. MS witness infiltration of immune cells including T-cells, B-cells, and peripheral monocytes across the blood brain barrier. Despite the development of highly effective treatments for relapse remitting MS, drugs that curtail MS progression remain elusive. Microglia, the brain- resident macrophages, play an important role in remyelination as they phagocytose damaged myelin during the early course of the disease. However, with age, microglia become chronically activated, lose phagocytosing capabilities, and contribute to MS progression. We are investigating the metabolic events that drive microglial dysfunction during chronic MS. Based on their inflammatory cues and activation states, immune cells undergo a shift between oxidative phosphorylation (OXPHOS; anti-inflammatory) and aerobic glycolysis (pro-inflammatory). We hypothesize that microglia also exhibit enhanced aerobic glycolysis during acute inflammation, and likely undergo 'metabolic deprivation' during chronic MS. We are therefore investigating the role of lactate dehydrogenase A (LDHA), a pyruvate-to-lactate converting enzyme, monocarboxylate transporter-4 (MCT-4), a lactate exporter, and extracellular matrix metalloproteinase inducer (EMMPRIN), a chaperone for MCT-4, in microglia. Notably, MCT- 4/EMMPRIN axis is critical to facilitate export of excess lactate in proinflammatory macrophages in MS. Using primary microglia and spinal cord tissues from peak disease in experimental autoimmune encephalomyelitis (EAE) mice, we found increase in the expression of LDHA, MCT- 4 and EMMPRIN in pro-inflammatory microglia. Further, we found siRNA-mediated knockdown of EMMPRIN to reduce TNF-α production in LPS-stimulated microglia. Using gene-specific knockdowns to perturb MCT-4/EMMPRIN axis, and utilizing the Seahorse assays, we are currently investigating the differences in pathways associated with aerobic glycolysis and OXPHOS between acute and long-term microglia activation in vitro. This will be corroborated by measuring a shift in metabolic pathways in microglia from the spinal

cords of chronic EAE (>D35) using high throughput flow-techniques and immunohistochemistry. We anticipate this work to unravel novel metabolic players/pathways for therapeutic targeting of MS progression

#65 Contribution of RNA Binding Protein Dysfunction to Neurodegeneration and Cortical Demyelination in Progressive Multiple Sclerosis

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Background: Gray matter demyelination, neurodegeneration, and RNA binding protein (RBP) dysfunction are pathologic features of the cerebral cortex in progressive multiple sclerosis (MS). Mislocalization of RBPs, including heterogeneous nuclear ribonucleoprotein A1 (A1), from the homeostatic nuclear location to the cytoplasm is a pathologic phenotype of neurons in the MS cortex. Because A1 is a central regulator of RNA metabolism in neurons, it can disrupt the function of other RBPs and impair multiple processes essential for neuronal function and survival. NeuN, or Rbfox3, is a neuron specific RBP that leads to synaptic dysfunction and excitotoxicity when depleted in neurons. As a feature of neurodegeneration in MS, we hypothesized that A1 dysfunction would alter NeuN expression in MS cortical neurons.

Methods: Using post-mortem cortical tissue from progressive MS patients and healthy controls, A1 dysfunction was quantitatively assessed in neurons using immunohistochemistry. A1 pathology was evaluated in normal appearing gray matter (NAGM) and demyelinated gray matter lesions (GML) by staining serial sections with proteolipid protein (PLP). MS cases were separated into two groups (high and low A1 pathology) based on the frequency of neurons with A1 pathology (i.e., nucleocytoplasmic mislocalization or nuclear depletion of A1) compared to control. Staining for NeuN, an RBP important for neuronal function, was used as a marker to quantify neurodegeneration (NeuN+ cells/mm^2).

Results: The rate of neurons with A1 pathology was significantly increased compared to control in the frontal and parietal cortices of MS NAGM ($p=0.013$) and GML ($p=0.017$). Paired analysis revealed no difference in the frequency of neurons with A1 pathology between GML and NAGM in MS. However, severity of A1 pathology positively correlated with GML load ($r=0.799$; $p=0.003$). A1 pathology correlated with loss of NeuN+ cells/mm^2 ($r=0.671$; $p=0.02$) and MS cases with high A1 pathology had fewer NeuN+ cells/mm^2 in cortical layers 3, 5, and 6 compared to MS cases with low A1 pathology and controls.

Conclusion: Neuronal A1 pathology correlates with increased GML load, a marker of more severe disease progression. In MS tissue with high A1 pathology, neurons in cortical layers 3, 5, and 6 were devoid of staining for NeuN, a protein critical to neuronal function. Dysfunction of A1 may contribute to decreased NeuN expression, a potential novel mechanism of neurodegeneration in MS.

Keyword: *multiple sclerosis, demyelination, neurodegeneration*

#82 Repeated Concussions in Mice Coupled with an Adjuvant Sensitize Myelin Basic Protein- Reactive T-Cells to Induce Central Nervous System Autoimmunity in Female SJL/J Mice

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that is characterized by demyelination and axonal injury. MS has been attributed to various genetic and environmental risk factors, one of which is the experience of repeated concussion injury in adolescence. However, the biological mechanisms of how concussion injury increases MS risk remain unknown. Here, we aimed to bridge this knowledge gap by investigating the effect of repeated, mild closed head injury (rmCHI) on the development of CNS autoimmunity in mice. First, we tested if two rmCHIs spaced one week apart could trigger spontaneous experimental autoimmune encephalomyelitis (EAE), the animal model of MS, in 2D2 myelin-specific T cell receptor transgenic mice (2D2 mice). We found that 2D2 mice in the rmCHI group did not exhibit increased EAE incidence compared to the sham-injured 2D2 group, suggesting that rmCHIs alone were insufficient to trigger CNS autoimmunity. Next, we tested if rmCHIs coupled with an immune boost (IB) consisting of Complete Freund's adjuvant (CFA) and pertussis toxin (PTX) could trigger CNS autoimmunity. We administered three rmCHIs in female SJL/J mice, each spaced one week apart. We injected mice with the IB after the second mCHI and PTX after the third mCHI. Two weeks after the third mCHI, mice were assessed for neurological dysfunction with the neurological severity scale (NSS) and myelin-specific T cell responses in the cervical lymph nodes, draining lymph nodes, and spleen with an enzyme-linked immunosorbent (ELISpot) assay. We also harvested the brains of mice to assess for peripheral immune cell infiltration with immunohistochemistry. We found that half of the mice in the rmCHI and IB group exhibited neurological deficits in anxiety (exit circle) and motor (beam balancing) tasks at the end-point. Moreover, ELISpot analysis in the draining lymph nodes and spleen revealed the presence of T cells specific against the immunodominant epitope of myelin-basic protein (MBP), but not other myelin antigens. CD45 immunostaining in the brains of these mice also revealed the presence of EAE-like lesions in the cerebellum and meninges. These findings suggest that an rmCHI with an IB can trigger CNS autoimmunity. Thus, our study establishes a preclinical model to test therapeutic agents that can delay or prevent autoimmunity post-CNS injury.

Keyword: *Multiple Sclerosis, Head Injury, Concussion*

#89 Exploring the Potential of Nebivolol as a Selective Modulator of Memory CD4+ T Cell Function: Insights into Alternative ßeta-2-Adrenergic Receptor Signaling Pathway and IL-17A Suppression

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Background: The sympathetic nervous system (SNS) modulates the function of T cells through the stimulation of beta-adrenergic receptors (β-AR). The beta-2AR has a classical G-protein

signalling pathway and an alternative pathway that can be selectively induced by biased agonists. However, the effect of β2-AR on a different subpopulation of T cells is not completely understood. In this study, we assessed the effect of nebivolol as a β2-AR biased agonist on human memory CD4+ T cells. We hypothesized that nebivolol is a potential drug to suppress IL-17A through the alternative β2AR cell-signalling pathway.

Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from healthy human participants. Memory CD4+ T cells were purified using immuno-panning methods. Samples were activated with Immunocult followed by nebivolol treatment for 4-5 days. The levels of IL-17A, IL-4, and IFN-γ were measured in the supernatants by ELISA. Flow cytometry was used to measure intracellular IL-17A, IFN-γ and proliferation by CFDASE. RORγt was measured by qPCR.

Results: Nebivolol reduced IL-17A secretion from activated PBMCs, in contrast, IL-4 and IFN-γ were not changed by the drug. When applied to activated memory CD4+ T cells (up to 98% purification), nebivolol inhibited IL-17A+ by intracellular cytokine staining as well as ELISA and decreased the expression of RORγt by qPCR. The effects of nebivolol on cellular proliferation and viability were not significant. Inhibition of PKA by H89 did not abrogate the effects of nebivolol on IL-17A secretion significantly.

Discussion: We demonstrated that nebivolol in a PKA-independent manner selectively inhibits IL-17A from human memory helper T cells and reduces the expression of RORγt as a main transcription factor for the Th17 subpopulation. The data suggests that nebivolol is a potential candidate for the treatment of chronic inflammatory diseases which exhibit elevated levels of IL-17 at the sites of inflammation.

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Keyword: *Nebivolol, Memory CD4+ T cells, Beta-2-Adrenergic Receptor, Biased Agonist, Interleukin-17A*

#100 Not-so-inflammatory development: Interleukin-1 cytokines regulate developmental microglial proliferation

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Microglia proliferate robustly in early postnatal life to reach a density that ensures appropriate function. Despite a growing appreciation for the importance of developmental microglial proliferation, the factor(s) that facilitate this proliferation remain unknown. Interleukin-1 (IL-1) cytokines—potent mediators of inflammation—are enriched during neurodevelopment and regulate microglial proliferation in other contexts. It may be that interleukin-1 cytokines, namely IL-1alpha and IL-1beta, have an undescribed capacity to regulate developmental microglial proliferation. In support of this, we found reduced microglial densities and altered proliferation in the developing brain and spinal cord of early postnatal IL-1alpha and IL-1beta knockout mice, though spinal cord densities normalized to control levels by postnatal day 30. To test whether IL-1 signaling directly or indirectly promotes microglial proliferation, we treated primary, serum-free microglia with IL-1alpha and IL-1beta alongside 28 other factors identified as potential mitogens from an exhaustive literature search. Interestingly, only colony-stimulating factors 1 and 2 (CSF-1, CSF-2), interleukin-34 (IL-34), and interleukin-3 (IL-3) boosted microglial proliferation. Therefore, we hypothesized that IL-1alpha and/or IL-1beta regulate developmental microglial proliferation by promoting mitogen release from astrocytes—a cell lineage that both secretes the identified mitogens and that alters its secretome in response to IL-1 signaling. Indeed, we found that astrocytes stimulated with IL-1alpha or IL-1beta released an unknown mitogen, or a group of mitogens, that dramatically boosted microglial proliferation in culture. Currently, we are validating the astrocytic expression of Csf1, Csf2, Il34, and Il3 in vivo via a combination of RNAscope and immunohistochemistry. In our preliminary analyses, astrocytic Csf1, but not Csf2, expression changes throughout development in line with microglial proliferation. Together, this work will identify the factor(s) that drive(s) microglia to initially establish their adulthood density and ensure proper functioning throughout life.

Keyword: *microglia, development, proliferation, Interleukin-1, astrocytes*

#101 Characterising the neuro-immune responses to peripheral inflammation

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Background: Immune cell infiltration into the brain during peripheral and central inflammation is a topic of contemporary interest in inflammation induced sickness behaviours. Recent studies indicate a strong relationship between increased proinflammatory cytokines and inflammation-induced behaviours, particularly in the context of immune-mediated inflammatory diseases. We aim to characterize the infiltrating immune cells and their respective cytokines along with brain resident cells including microglia, neurons and astrocytes in peripheral and central inflammation following administration of a TLR7 agonist.

Methods: Mice were treated with topically administered Aldara (containing TLR7 agonist Imiquimod), or control cream every 24 hours for

3 days. Flow cytometry was performed on neural single cells to identify, characterize, and quantify infiltrating immune cells and brain resident cells. Intracellular staining was used to assess cytokine production from cells. Confocal imaging was done to understand astrocyte and microglia biology. qPCR data from brain parenchyma was also analysed for chemokines.

Results: Topical Aldara treatment results in the infiltration into the brain of both lymphoid and myeloid immune cell population. A subset of these recruited lymphoid population is regulatory in nature e.g., Th17. Resident neural cells (microglia, neurons, and astrocytes) are also altered in term of both quantity and reactivity during the inflammation.

Conclusions: Treatment with Aldara results in a potent inflammatory response in the brain characterized by infiltration of immune cells. These recruited immune cells might be a source of intracerebral pro-inflammatory cytokines and chemokines that might impact upon resident neural cells and neural connectivity. Further experiments are needed to understand the mechanisms of immune cell entry into the brain and to explore the fate of these cells and their possible role in sickness behaviours.

#110 TRIM32-mediated type I interferon signaling causes tactile hypersensitivity in mice lacking translational repressor 4E-BP1 in Nav1.8-positive sensory neurons

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mTOR is a highly evolutionarily conserved serine/threonine kinase that regulates cell homeostasis through key cellular processes, including cell growth and proliferation, mRNA translation, autophagy, and cytoskeleton organization. mTOR is present in two structurally and functionally distinct multiprotein complexes: mTORC1 (mTOR Complex 1) and mTORC2. The activity of mTORC1 is increased in several monogenic disorders that are co-diagnosed with high rates of autism (such as fragile X, Rett and Phelan-McDermid syndromes) and was shown to promote tactile hypersensitivity. The specific mechanism by which increased mTORC1 promotes hypersensitivity remains unknown. mTORC1 regulates the rate of eIF4E-dependent mRNA translation via inhibition of translational repressor 4E-BP1. Mimicking the activation of the mTORC1-dependent translation by whole-body deletion of 4E-BP1 in mice produces robust tactile hypersensitivity. To understand the underlying mechanism, we selectively ablated 4E-BP1 in Nav1.8-positive sensory neurons (4E-BP1 cKO). We then assessed behavioral phenotypes and DRG neuron excitability, as well as performed translating ribosome affinity purification (TRAP) to study differentially expressed genes (DEGs) in sensory neurons. Our behavioral experiments

demonstrated that 4E-BP1 cKO mice exhibit basal tactile hypersensitivity but no thermal phenotypes. Profiling gene expression in sensory neurons lacking 4E-BP1 (using TRAP approach) revealed changes in pathways involved in antiviral responses and mitochondrial activity. Follow up experiments showed TRIM32-mediated increase in type I interferon signaling. Blocking interferon receptors reversed the tactile hypersensitivity and neuronal excitability in 4E-BP1 cKO mice. Our study demonstrates the central role of eIF4E-dependent translational control in Nav1.8-positive sensory neurons in regulating mechanical sensitivity. Moreover, our results indicate that ablation of 4E-BP1 in these neurons increases the translation of TRIM32 which promotes interferon-mediated tactile allodynia.

Keyword: *type I interferon, nociceptors, mTOR, inflammation, pain*

#112 Modulation of Neuropathic Pain in Multiple Sclerosis by Spinal Microglia and Sphingosine-1-Phosphate Receptor S1PR1

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Neuropathic pain afflicts well over half of people living with multiple sclerosis (MS), but current treatments but do not address MS associated neuropathic pain (MSNP). This is due in part to an incomplete picture of its underlying mechanisms. The pathophysiology of MS includes proinflammatory microglial activation and previous studies suggest that microglia mediate hypersensitivity in models of peripheral neuropathic pain; however, their contribution to central neuropathic pain including MSNP, remains unclear. To study MSNP, we induced experimental autoimmune encephalomyelitis (EAE) by the subcutaneously injecting of MOG^{33-55} (in complete Freund's adjuvant, CFA) into the hind flank of adult C57Bl/6 mice. As expected, we found that EAE but not CFA alone caused persistent mechanical (von Frey) and cold (acetone) hypersensitivity at the hindpaw. In our first set of studies, spinal microglia depletion was achieved with intrathecal (i.t.) injection of liposome-encapsulated clodronate (LEC). LEC did not change cold hypersensitivity but did attenuate mechanical hypersensitivity at 72-hours post injection. Interestingly, cold hypersensitivity appears to be mediated through nonpeptidergic C-fibers in the periphery as demonstrated by depletion of MrgprD+ afferents leading to reduced cold but not mechanical hypersensitivity. Our second set of studies were based in part on reports that the sphingosine-1-phosphate receptor type 1 (S1PR1), a Gi-coupled GPCR, is an emerging target for the treatment of persistent pain that is expressed on microglia. The S1PR agonist/functional antagonist fingolimod, an FDA-approved disease modifying agent for MS, reduces pain-like behaviors in models of inflammatory and neuropathic pain including EAE. However, the site and mechanism of antiallodynic action of fingolimod, remains unknown. To begin to address these questions, EAE mice received i.t. injection of S1PR agents fingolimod, SEW2871 (an S1PR1-selective agonist), NIBR0213 (an S1PR1-selective antagonist), fingolimod + NIBR0213, or vehicle. Only fingolimod alone or SEW2871 alone attenuated mechanical and cold hypersensitivity, and the former was blocked by NIBR0213, suggesting that pharmacological agonism at spinal S1PR1 reduces MSNP. We conclude that spinal S1PR1 and microglia are unique targets for development of future pharmacotherapies to

treat MSNP, and future studies are planned to specifically target S1PR1 on spinal microglia.

Keyword: *Pain, Microglia, Multiple Sclerosis, Sex Differences, S1PR*

#135 Human embryonic stem cell - derived oligodendrocyte progenitor cells migrate and differentiate into myelinating oligodendrocytes in the brain of experimental multiple sclerosis

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Introduction
Cell therapy in multiple sclerosis (MS) has utilized to date only immune-modulatory and trophic therapeutic properties of transplanted cells. To date, no cellular platform has proved effective for true regenerative-remyelinating cell therapy in a clinical-relevant animal model of chronic-progressive MS. We developed the transplantation of human embryonic stem cell (hESC) -derived oligodendrocyte progenitor cells (OPC). We utilized the chronic-progressive Experimental Autoimmune Encephalomyelitis (EAE) model in Biozzi mice, as the most clinical-relevant model for chronic MS.

Design & Methods
hESC-OPC were transplanted into cerebral white matter tracts of newborn mice, and of adult mice with chronic EAE. Human cells were identified in the host brain by immune-fluorescence for human specific markers, and by using transgenic human cells expressing Red Fluorescent Protein (RFP) under the Myelin-basic-protein promotor. Histochemistry was performed to determine the state of myelination, and double immunofluorescent stains were performed to assess the differentiation of transplanted cells.

Results
In initial studies, we showed that in the chronic phase of Biozzi-EAE mice develop demyelinated plaques with pathological and immunological resemblance to chronic MS. Then, we examined the potential of hESC-OPC, collected at several stages of development to migrate and differentiate in the brain of newborn mice. A chosen hESC-OPC cell platform was then transplanted into Biozzi-EAE mice at the chronic phase of disease. We observed large distance migration of transplanted cells in the chronically demyelinated and inflamed host brain, and their differentiation into myelinating oligodendrocytes. The differentiation of transplanted hESC-OPC into MBP+ oligodendrocytes was further validated by transplanting RFP-MBP expressing cells. Finally, hOPC-transplanted mice exhibited clinical improvement, whereas sham-transplanted mice continued to deteriorate.

Discussion
This is the first observation of potentially true regenerative cell therapy in MS.

Keyword: *Multiple Sclerosis, Cell Therapy*

#145 Role of astrocyte metabolism during acute and chronic neuroinflammation

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Multiple sclerosis (MS) is an autoimmune disease characterized by immune cell infiltration, demyelination and neuronal cell loss. Glial cells such as oligodendrocytes, microglia and astrocytes are of key interest in the development and progression of neuroinflammation. In this context, astrocytes have been shown to play a crucial role in maintaining central nervous system (CNS) homeostasis and, in particular, in

supporting neuronal metabolism. However, numerous studies have demonstrated impaired astrocyte function in chronic inflammation, which contributes to disease progression. In this context, the metabolic support of neurons by astrocyte-derived lactate fails for yet unknown reasons. Recent research has demonstrated changes in astrocyte metabolism resulting in increased release of neurotoxic mediators and decreased neurotrophic support during chronic neuroinflammation in experimental autoimmune encephalomyelitis (EAE), the animal model of MS. Using ex vivo analysis of metabolic dependencies, high dimensional flow cytometry and RNA-seq analysis we are investigating the underlying alterations of astrocyte metabolism, causing dysregulation of CNS homeostasis and disease progression. Overall, we aim to unravel the underlying mechanisms whereby persistent neuroinflammation alters astrocyte metabolism, with the ultimate goal of developing new therapeutic strategies for progressive stages of MS where current treatment strategies are limited.

Keyword: *multiple sclerosis, astrocytes, metabolism*

#146 Dendritic pathology in a mouse model of MS

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Multiple sclerosis (MS) is classically considered an inflammatory demyelinating disease of the white matter (WM). However, evidence for grey matter (GM) pathology is accumulating. MS patients show demyelinated lesions within the GM, alongside a loss of neurons, and synapses. Despite increasing efforts to understand the neurodegenerative nature of this disabling disease, the role of dendrites is yet to be elucidated.

In this project, we aim to characterize dendritic pathology on an anatomical and molecular level in a mouse model of MS, referred to as experimental autoimmune encephalomyelitis (EAE). Within this model, demyelinating lesions (i) coincide with a marked infiltration of microglia, (ii) occur most frequently within the lumbar spinal cord, and (iii) expand from the pial surface into the white matter towards the GM. Given this information, we focus on dendrites originating from motor neurons (MNs) located in the lumbar ventral horn, which extend into the (WM).

So far, genetic tracing of motor neurons and immunofluorescence analysis of dendrites revealed a general loss of dendritic density in the spinal cord of EAE mice compared to wild type mice. Interestingly, the number of MNs remains unchanged, indicating that the loss of dendrites is not merely due to the loss of neurons, but rather an independent phenomenon. We hypothesize that the widespread inflammation found throughout the EAE spinal cord could influence dendrites via soluble immune mediators like IFN-gamma. Alternatively, elevated glutamate levels could lead to excitotoxicity and dendritic damage via NMDA and AMPA receptors.

To investigate these potential molecular mechanisms of dendritic pathology, we developed a novel neuronal CRISPR-KO workflow and, additionally, use an inducible transgenic line overexpressing the calcium-impermeable AMPA subunit GluA2. Up to this point, neither the depletion of Ifngr1, nor blocking of calcium-influx via AMPA receptors rescued the dendrites. Further mediators, e.g. NMDA-R, still need to be evaluated.

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Keyword: *Multiple Sclerosis, Spinal Cord, Grey Matter, Dendrites, CRISPR*

#148 The Imiquimod Model: Transcriptomic and Blood-Brain Barrier changes following peripheral and central Imiquimod TLR7-induced neuroinflammation.

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Background & Aims

Systemic inflammation is associated with CNS changes and subsequent behavioural effects known as sickness behaviours (SB: mood changes, fatigue, social withdrawal, and cognitive impairment). They can increase disease burden on the individual, their family and socioeconomic community, together with mediating a significantly negative impact upon treatment adherence, response and outcome in systemic disease. This work focuses on peripheral and central characterisation of inflammation in the Imiquimod (IMQ) mouse model and links to SB. Previously, we demonstrated IMQ enters the brain directly , resulting in a central as well as peripheral TLR7-stimulated inflammation. We investigated markers of inflammation, blood brain barrier (BBB) changes, and infiltrating leukocyte populations (ILP) to brain parenchyma.

Methods

IMQ cream (62.5mg/dose) was applied topically on the shaved mouse back for three consecutive days. Bulk RNA sequencing was performed on whole brain hemisphere homogenate. Intravenous injection of Evan's Blue Dye (EBD) was completed to assess BBB integrity. Changes to BBB tight junction (TJ) associated proteins and enzymes were investigated using western blots. Brain regionality of ILP was investigated using CD3+ immunofluorescence.

Results

IMQ topical application induced a psoriasis-like dermal reaction. PCA analysis found distinct groupings of control and treatment groups. Significant gene analysis found 2198 upregulated and 585 downregulated genes. EBD was present in treatment brains and not in controls showing an impairment in BBB integrity. Increased TJ protein and enzyme expression was found in brain parenchyma of treatment groups. A global infiltration of CD3+ cells was observed into the brain parenchyma with no obvious brain regionality.

Conclusion & Future Directions

The IMQ model presents a mild neuroinflammatory model with changes to BBB and ILP infiltration to brain parenchyma. Bulk RNA sequencing has shown transcriptomic differences between control and treatment groups. These transcriptional changes will be further explored through different bioinformatic analysis packages. Future experiments will investigate the functional BBB integrity by exploring transendothelial electrical resistance. Electrophysiology experiments will describe synaptic activity changes and behavioural tests including open field and elevated plus maze will provide a fuller picture relating to SB. Spatial

biology of this neuroinflammatory environment is planned to be investigated with CosMx and Codex. Building on current findings with the above future experiments will aid further characterisation of the neuroinflammation present in the IMQ model and its downstream consequences. This may allow identification of therapeutic targets to attenuate SB.

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Keyword: *Neuroinflammation, Sickness Behaviours, Depression, Neuroimmunology, Psychoneuroimmunology*

#149 Exogenous cholesterol promotes phagocytosis of myelin debris in vitro

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Multiple sclerosis (MS) is an inflammatory and progressive disease of the central nervous system (CNS), characterized by immune cell infiltration, demyelination, and neurodegeneration. Demyelination leads to the elaboration of myelin debris, which prevents remyelination by inhibiting the differentiation of oligodendrocyte progenitor cells into mature and myelinating oligodendrocytes. Thus, promoting the phagocytosis of myelin debris would be a promising strategy to enhance remyelination and repair in the CNS following a demyelinating attack. Our aim was to promote the clearance of myelin debris by murine bone marrow-derived macrophages (BMDMs) and human microglia. To do so, we tagged CNS myelin with pHrodo™, a pH-sensitive dye that fluoresces at low pH, such as would be found in the phagolysosome of phagocytic cells. We treated cells with various compounds, including LPS, cholesterol, and cytochalasin D. Following treatment, media was supplemented with pHrodo-tagged myelin, and cells were imaged six hours later. First, using the phagocytosis inhibitor cytochalasin D as a negative control, we confirmed that the pHrodo fluorescent signal represented phagocytosis, as no fluorescence was observed in cells treated with the inhibitor. We also found that the number of BMDMs and human microglia engaging in phagocytosis of myelin debris was strongly upregulated by treatment with exogenous cholesterol. Lastly, we observed that treatment with cholesterol significantly enhanced viability of BMDMs. Future work will elucidate the mechanisms through which cholesterol promotes phagocytosis, including its involvement in regulating the LXR pathway and lipid homeostasis within the CNS. We will also strive to translate these findings into an in vivo model of multiple sclerosis, to determine whether enhanced phagocytosis of myelin debris through modulation of lipid pathways is a promising strategy to ameliorate disease.

Keyword: *Neuroinflammation, Phagocytosis, Myeloid cells*

#153 Immunoregulatory properties of glial MIF in the context of autoimmune CNS inflammation

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Multiple sclerosis is a chronic autoimmune disease of the central nervous system (CNS). After acute lesions, the resolution or chronification of tissue damage is mainly determined by the interplay between CNS-resident cells and infiltrating immune cells shaping either an anti- or a pro-inflammatory microenvironment. In recent years, inflammation-modulating factors governed by glial cells, such as astrocytes and microglia, have been demonstrated to promote or attenuate disease progression. One cytokine recently shown to be involved in the crosstalk of glial cells is the macrophage migration inhibitory factor (MIF). As MIF is highly expressed in human active MS lesions, the cytokine may evoke an inflammatory response in MIF-sensing cells due to the expression of CD74, the receptor of MIF. Indeed, the interaction of microglia and astrocytes controlled by the MIF-CD74 axis is tightly regulated over the course of autoimmune CNS inflammation as exemplified by our findings in the experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Here, we will determine the spatiotemporal expression of MIF and its receptor CD74 as well as the effects of MIF on responder cells in vitro and in vivo. The impact of MIF in EAE will be examined using in vivo CRISPR-Cas9-mediated gene editing as well as intranasal treatment approaches. In that way, we will decipher a potential novel druggable target for acute and chronic stages of neuroinflammation, for which current therapies are limited.

Keyword: *glial cells, autoimmune CNS inflammation, experimental autoimmune encephalomyelitis, astrocytes, macrophage migration inhibitory factor*

#161 NIK in CX3CR1 expressing cells drives experimental autoimmune encephalomyelitis

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NF-kB-inducing kinase (NIK) is a primary stimulator of the non-canonical NF-kB signalling pathway, which regulates several aspects of the immune system. During an immune response, inflammatory mediators cause an accumulation of NIK, which subsequently activates the pathway. This in turn results in the transcription of multiple downstream genes involved in host defence. Germline deletion of NIK in mice results in resistance to experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. However, the mechanism behind this resistance is still unclear, and cell-specific deletions are required to investigate the role of NIK in autoimmune inflammation.

Both microglia and macrophages have been shown to play an important role in neuroinflammation. Therefore, we decided to investigate the role of NIK in these cells by crossing mice that allow the conditional deletion of NIK with a mouse line expressing Cre-recombinase under the CX3CR1 promoter ($NIK^{\Delta CX3CR1}$). In these mice, NIK is deleted in all $CX3CR1^{+}$ cells, which include microglia, macrophages, and a subset of dendritic cells. A second strain, $NIK^{\Delta MG}$, is inducible and requires tamoxifen treatment, and deletion is restricted to the microglia.

We found that these $NIK^{\Delta CX3CR1}$ mice are completely resistant to EAE and do not develop any clinical symptoms. They have no infiltrating

immune cells in the CNS and fewer activated microglia. Furthermore, we see a reduction of activated T-cells in the draining lymph nodes before the onset of the disease. However, this phenotype is reversed when NIK is deleted specifically in microglia. These $NIK^{\Delta MG}$ mice show no difference in CNS infiltrating immune cells or microglia activation at the peak of disease compared to littermate controls. This data suggests that the protective effect seen in $NIK^{\Delta CX3CR1}$ is due to the deletion of NIK in other peripheral CX3CR1-positive cells and not microglia.

Taken together, our data suggest that NIK in peripheral $CX3CR1^{+}$ cells plays an important role in the development of autoimmune neuroinflammation by affecting T-cell priming or migration.

Keyword: *Autoimmunity, EAE, Neuroinflammation*

#194 Human embryonic stem cell – derived astrocytes (AstroRx®) possess trophic properties that may overcome remyelination failure

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Introduction

Remyelination failure in chronic multiple sclerosis is caused in part by lack of sufficient removal of myelin debris, lack of OPC differentiation and insufficient inflammatory activity. AstroRx® is a cell therapy product comprised of human astrocytes derived from embryonic stem cells. AstroRx® support the survival of neurons and is tested in clinical trials for the treatment of Amyotrophic Lateral Sclerosis (ALS). AstroRx® was shown to possess neurotrophic properties, such as clearing excessive glutamate, reducing oxidative stress, and secreting various neuroprotective factors. We studied here their potential value in CNS demyelinating diseases.

Design & Methods

Co-culture assays were performed to evaluate the effect of AstroRx® on (1) murine microglia phagocytotic activity, using CFSE-labeled myelin debris; (2) murine NG2+ OPC proliferation (by BrdU incorporation), differentiation into O1+ oligodendrocytes and their survival; (3) activation, proliferation, and death of murine lymphocytes, by staining for CD25, BrdU, CD3, CFSE, Annexin-V and Propidium Iodide.

Results

Both AstroRx® and their conditioned medium significantly enhanced microglial phagocytosis of CFSE-labeled myelin debris. AstroRx® promoted OPC differentiation into mature oligodendrocytes, AstroRx®, had no effect on activation and proliferation of CD3+ T cells, by CD25 and BrdU staining. However, CFSE staining indicated that AstroRx®, significantly reduced lymphocyte apoptosis and necrosis and increased the number of cell divisions in lymphocytes.

Discussion

AstroRx® possess powerful trophic properties by which they improve clearance of myelin debris, promote oligodendrocyte differentiation, and maintain neuroinflammation, which are critical in overcoming remyelination failure in multiple sclerosis. We are currently studying their clinical and pathological effects in the chronic phase of experimental autoimmune encephalomyelitis.

Keyword: *Cell Therapy*

#198 Preformed fibrils induced neurodegenerative disease relevant transcriptomic profile in the in vitro human iPSC CNS model system

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Protein abnormalities are a common etiology shared across multiple neurodegenerative diseases including Amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Alzheimer's disease (AD). Toxic protein aggregates can influence a plethora of molecular mechanisms, such as metabolic and inflammation related pathways which are known to induce neurodegeneration by disrupting not only neuronal function but also dysregulating glial cell function. We have previously shown that acute incubation of synthetic preformed fibrils (PFF), the disease-relevant protein aggregates, with an in vitro human iPSC triculture system composed of astrocytes, neurons and microglia altered the transcriptome as well as induced the release of PFF-specific cytokines and chemokines. In this follow-up study, we conducted a transcriptomic connectivity analysis and identified a positive correlation between the differentially gene expression profiles of the PFF-stimulated tricultures and publicly available transcriptomic profiles of 128 brain regions collected from over 1000 patients with AD, ALS or PD. Subsequent tissue-level analysis revealed brain region specific association with each of the PFF conditions. Of note, the tau-PFF condition showed the highest correlation with the temporal lobe of patients with AD coinciding with a familiar pattern of AD progression. In contrast, the α-synuclein-PFF condition displayed high correlation with the medulla oblongata of PD tissues, a known affected area in patients with early PD. Together, this study demonstrated that the tau and α-synuclein protein aggregates induced a disease relevant transcriptomic profile in the human cell-based in vitro CNS model system, and this in turn can be used as a beneficial tool in understanding cellular changes relevant to disease progression.

Keyword: *PFF, Tau, Synuclein, Glia, Neurodegenerative disease*

#205 Unraveling the Mechanisms of Inflammation and Pain Responses Mediated by IL-1 Receptor Isoforms

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INTRODUCTION

The International Association for the Study of Pain describes pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage." It is one of the primary reasons for seeking medical consultation, and approximately 20% of adults worldwide experience it[1]. In individuals with inflammatory autoimmune such as multiple sclerosis (MS), rheumatoid arthritis (RA), and osteoarthritis (OA), chronic pain is present in more than 50% of patients. Pain is transmitted by nociceptors to the spinal cord and the brain via dorsal root ganglions (DRGs), where the cell bodies of sensory neurons are located[2]. The proinflammatory cytokine interleukin-1beta (IL-1beta) can trigger both inflammatory and pain responses.

OBJECTIVE

Identify the mechanisms mediating the inflammatory and pain responses induced by IL-1beta.

METHODS

Injections of recombinant IL-1beta were performed in wild-type mice, mice with a cell-specific deletion or restoration of the Il1r1 gene, and mice globally lacking IL-1R1. We then performed pain behavioral tests and carried out post-mortem analysis of the types of neurons expressing IL-1R1. Finally, we performed a 5'RACE-PCR and Western blot respectively on the total mRNA and the total proteins of the DRGs.

RESULTS

Our work has shown that in DRGs, IL-1R1 is expressed exclusively in a nociceptor subtype that expresses TRPV1. Deletion of the Il1r1 gene specifically in these nociceptors prevented the development of mechanical pain in mice with experimental autoimmune encephalomyelitis (EAE), without affecting other clinical signs of these diseases. Moreover, restoring Il1r1 expression exclusively in these nociceptors in otherwise IL-1R1 knockout mice resulted in full restoration of pain behaviors in these animal models. We also detected from total RNA extracted from DRGs the presence of a transcription initiation site for a novel Il1r1 mRNA subtype, as described by Qian et al.[3], and confirmed by Western blotting the expression of a truncated form of the IL-1R1 protein, that we called τIL1R1. Indeed, we detected a distinct and unique band in the protein extract of DRGs with a lower molecular weight compared to bEndD.3, a brain endothelial cell line.

CONCLUSION

IL-1beta triggers both the inflammatory and pain responses independently. This pain response is mediated by $TRPV1^{+}IL1R1^{+}$ nociceptors. These nociceptors may express a shortened isoform of IL-1R1 causing the pain response. In the long run, this project could lead to the identification of new therapeutic targets, thereby reducing pain in patients suffering from chronic inflammatory diseases.

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Keyword: *IL1R1, IL-1beta, DRG, Pain, TRPV1*

T cells in Neurological diseases

#19 MicroRNA-150 controls experimental autoimmune encephalomyelitis by modulating regulatory T cell differentiation and function

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MicroRNAs are small non-coding RNA molecules that fine-tune diverse biological processes and are often found to be dysregulated in diseases, such as multiple sclerosis (MS). MS is an immune-mediated disease of the central nervous system characterized by demyelination, axonal loss and neurodegeneration. We have previously shown microRNA-150 (miR-150) levels to be elevated in cell-free cerebrospinal fluid (CSF) of MS patients compared to controls. We aimed to investigate the physiopathological function of miR-150 in vivo by generating miR-150 knock-out (KO) and knock-in (KI) mice using CRISPR/Cas9 technology. To specifically interrogate the role of miR-150 upon inflammation of the central nervous system (CNS), we induced experimental autoimmune encephalomyelitis (EAE), a mouse model for MS, in these newly generated mice. After induction of EAE, miR-150^{KO} mice developed milder disease compared to WT littermate controls while miR-150^{KI} mice presented with exacerbated EAE. Disease amelioration in miR-150^{KO} mice was accompanied by decreased infiltration of CD4^{+} T cells in the central nervous system compared to WT and KI mice, as well as increased FoxP3^{+} regulatory T (Treg) cells in inguinal lymph nodes at disease priming stage. We demonstrated that Treg cells were fundamental for EAE amelioration in miR-150^{KO} mice, as their partial depletion during EAE priming stage in miR-150^{KO} FoxP3^{DTR} mice restored disease incidence and severity to the levels observed in WT and KI mice. Transcriptomic profiling of CD4^{+} T cells isolated from miR-150^{KO} mice revealed upregulation of genes associated with Treg cell function, but also reduced gene translation and autophagy, as compared to WT and particularly KI cells. The role of miR-150 in affecting the fate of CD4^{+} T cells was further supported by the grater tendency of miR-150^{KO} CD4^{+} T cells to differentiate into Treg cells in-vitro. In conclusion, miR-150 deficiency favored considerably milder CNS inflammation by promoting differentiation of a more anti-inflammatory CD4^{+} T cell repertoire.

Keyword: *MicroRNA, Multiple sclerosis, Treg*

#76 Role of Intracellular Cell Adhesion Molecule 1 on T cells in MS

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Multiple sclerosis (MS) is a chronic inflammatory and degenerative disorder of the central nervous system (CNS) that is the leading cause of neurological disability in young adults. Neuroinflammatory processes cause acute and chronic neuroglial injury and impair CNS repair mechanisms in people with MS, causing irreversible deficits in sensory, motor and/or cognitive functions. Understanding how to protect neuroglial cells from immune-mediated damage is therefore crucial to address progression in MS.

CNS-infiltrating pro-inflammatory activated T cells are pivotal immune mediators in MS and are known to both contribute to neuroglial injury and to the inflammatory MS lesion environment. Intercellular adhesion molecule-1 (ICAM-1) expressed by myeloid cells and endothelial cells has many roles in both physiological and pathological conditions for the formation of the

immune synapse with T cells and for leukocyte trafficking. While it has been shown that ICAM-1 expressed on T cells can also act as a co-stimulatory molecule that promotes T cell activation and proliferation, the role of ICAM-1+ T cells in MS pathophysiology and disease progression remains unclear. As our lab has observed that T cells from MS patients and EAE mice differentially express ICAM-1 compared to healthy controls, we hypothesize that ICAM-1+ T cells promote neuroinflammation and play a pathogenic role in MS.

To elucidate the role of ICAM-1 signaling on T cells in MS and its animal model experimental autoimmune encephalomyelitis (EAE), our lab has generated a novel transgenic line to conditionally ablate ICAM-1 on CD4 and CD8 T cells (CD4cre:ICAM-1^{flfl} mice). Following MOG_{35-55} active EAE induction, ablation of ICAM-1 on T cells resulted in a mild reduction in peak scores only in females, but was associated with a marked improvement of EAE clinical scores in the chronic phase of the disease in both sexes. Deletion of ICAM-1 on T cells resulted in reduced serum neurofilament light chain (sNfL) levels, reduced activation of microglia/myeloid cells, and limited CNS infiltration of pathogenic immune cells after peak in active EAE. In addition, blocking ICAM-1 on human primary T cells prior to co-culture with human primary oligodendrocytes resulted in improved oligodendrocytes survival.

These data suggest that ICAM-1+ T cells sustain deleterious neuroinflammatoy processes associated with EAE progression in the chronic phase and with CNS neuroglial injury in vitro and in vivo.

Keyword: *Multiple Sclerosis, T cells, ICAM-1, EAE*

#95 Infiltrating CD8+ T cells exacerbate Alzheimer's disease pathology in a novel 3D human neuroimmune axis model

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Peripheral immune cells have been proposed to play a role in Alzheimer's disease (AD) pathology by infiltrating the brain and interacting with resident cells. However, the potential disease-modifying role of human peripheral immune cells in the AD brain remains unclear. Here, we describe a novel three-dimensional (3D) human neuroimmune axis model comprised of human stem cell-derived neurons, astrocytes, and microglia, together with human peripheral immune cells. We used this neurimmune axis model to examine the infiltration of peripheral immune cells into AD versus control neural-glial cell cultures. We observed a dramatic increase in the number of human T cells (but not B cells) selectively infiltrating into AD cultures versus control, regardless of the presence or absence of microglia. Human monocyte infiltration into AD neuron/astrocyte cultures was also greater than that observed in control cultures. However, the presence of microglia equalized the rates of monocyte infiltration into the AD and control cultures. Infiltration of CD8+ T cells into AD cultures led to enhanced activation of microglia and exacerbated levels of neuroinflammation and neurodegeneration. Using single-cell RNA-

sequencing, we identified that infiltrating T cells into AD cultures led to induction of interferon-gamma (IFNg) and neuroinflammatory-associated pathways in glial cells. In addition, we discovered key roles for the C-X-C motif chemokine ligand 10 (CXCL10) and its receptor, CXCR3, in regulating T-cell infiltration into the neurimmune axis model AD cultures. Modulating this T cell CXCL10/CXCR3 signaling pathway largely prevented neuronal damage in AD neural-glial cultures. Thus, CXCL10-induced, infiltrating CD8+ T cells and microglia are important mediators of brain neurodegeneration in an AD human cellular model. Our novel 3D human neuroimmune axis model can now be employed to explore the potentially detrimental or beneficial roles of peripheral immune cell infiltration into the brain and guide novel diagnostics and therapeutics for AD.

Keyword: *T cells, CD8, Microglia, Neuroinflammation, Neurodegeneration*

#125 Identification of essential modules regulating T cell migration to the central nervous system in multiple sclerosis

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Multiple sclerosis (MS) is initiated by the infiltration of autoreactive T cells into the CNS, yet we lack a comprehensive understanding of the signalling pathways that regulate this process. We conducted a genome-wide in vivo CRISPR screen in a preclinical MS model and identified 5 essential brakes and 18 essential facilitators of T cell migration to the CNS. While the transcription factor ETS1 limits entry to the CNS by controlling T cell responsiveness, three functional modules, centred around the adhesion molecule α4-integrin, the chemokine receptor CXCR3, and the GRK2 kinase, are required for CNS migration of autoreactive $CD4^+$ T cells. Single-cell analysis of T cells from patients with MS confirmed that the expression of these essential regulators correlates with the propensity of $CD4^+$ T cells to reach the CNS. Our data thus reveal the key molecules and modules that govern the fundamental step in the induction of MS lesions.

Keyword: *Multiple sclerosis, Experimental Autoimmune Encephalomyelitis, T cell migration, CRISPR Screen, multiphoton microscopy*

#133 CNS myeloid cell-driven inflammation is modulated by adoptive Treg transfer

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Chronic central nervous system (CNS) inflammation is characterized by myeloid cell activation that is not sufficiently targeted by current therapeutic approaches. We here investigate the interaction of adoptively transferred regulatory T cells (Tregs) with brain-resident cells and the mechanisms of their effect on the inflammatory micromilieu. Tregs ameliorate symptoms of ongoing experimental neuroinflammation if administered in symptomatic mice. In the inflamed CNS, T effector cells display a constant screening behavior of target cells including Tregs. Those Treg-T effector cell contacts are frequent but short and transient, excluding them as a form of cell-cell suppression. Tregs preferentially form long-lasting, stable contacts with CNS myeloid cells exerting a strictly antigen-specific suppressive effect. Using a combination of RNA sequencing and detailed mechanistic analyses, we were able to demonstrate that transforming

growth factor beta (TGF-β) secreted by Tregs resulted in reduced T effector function mediated by CNS myeloid cells. Single cell RNA sequencing from human cerebrospinal fluid (CSF) cells of patients with multiple sclerosis (MS) showed a downregulation of TGF-β-dependent pathways in inflammatory CSF monocytes, indicating a proinflammatory dysregulation of Treg-myeloid cell interaction in CNS inflammation. A CXCL10-centered proinflammatory protein cluster in the CSF identifies multiple sclerosis patients with higher neuronal damage. Altogether, we provide evidence that proinflammatory CNS myeloid cells could be therapeutically targeted by Treg-based strategies.

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- [3] PMID 34702947

Keyword: *Treg, Microglia, Multiple Sclerosis, CSF, CXCL10*

#139 Phenotyping of CD4 CTL subsets that develop due to different chronic antigenic triggers using single cell multiomics

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Cytotoxic CD4 T cells (CD4 CTL) are a subset of immune cells that have been shown to contribute to disease progression of MS patients. This immune cell subset is not present in all individuals, but is thought to develop due to chronic antigenic stimulation. These cells are therefore commonly found in aged individuals, persons with latent viral infections, and patients suffering from autoimmune diseases. How exactly these cells develop remains unclear, but existing literature suggests that the type of antigenic trigger and the micro-environment in which CD4 CTL develop, dictates their phenotype. As CD4 CTL are in general only present in small absolute numbers, isolation of sufficient numbers of cells to study their heterogeneity at a phenotypical and functional level can be challenging. Recently developed techniques focused on analysis of single cells now make it possible to study even rare cell subsets like CD4 CTL in great detail. In this study, single cell multiomics technology is applied to study the phenotype and development of CD4 CTL, by combining information from single cell RNA sequencing with analysis of surface proteins expressed by the same cell. By comparing cells derived from multiple sclerosis (MS) patients (autoimmune trigger) with cells derived from healthy controls (HC) with a latent CMV infection (viral trigger), we determined whether differences exist between CD4 CTL induced by different antigenic triggers. We show here that the CD4 CTL population can be separated into distinct subsets with divergent expression of molecules associated with cytotoxicity based on expression of CD28, Eomes, GZMA, GZMB, GZMK, and IFN-γ. When comparing the profile of $CD4^+CD27^-$ T cells (which are regarded as precursors of CD4 CTL) between CMV seropositive HC and MS patients, the phenotype was strikingly similar identifying 4 different full blown $CD28^-$ CD4 CTL subsets. In contrast, CMV seronegative MS patients had reduced numbers of these CD4 CTL clusters, indicating that MS as such is not sufficient to induce cytotoxicity in $CD4^+CD27^-$ cells.

Overall our data indicate that CD4 CTL are an intrinsically heterogeneous population, and confirm that latent CMV infection is a major driver of $CD28^-$ CD4 CTL expansion. While

functional differences in pathogenicity remain to be elucidated, the work presented here provides a framework for the identification of novel targets for the diagnosis, treatment, and prevention of progression of MS.

Keyword: *CD4 CTL, multiple sclerosis, cytomegalovirus, single cell multiomics*

#175 Meningeal lymphatic endothelial cell regulation of T cell function and migration

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NIH/NICHD

While the CNS parenchyma is generally cloistered from lymphocytes, the meninges are home to many patrolling immune cells. T cells are necessary for development, healing, and infection clearance in the CNS. However, during traumatic brain injury- or multiple sclerosis-associated inflammation aggregation of lymphocytes in the meninges of mammals can have harmful effects, and failure to resolve neuroinflammation may lead to chronic sequelae. Lymphatic endothelial cells (LECs) serve as an important regulator of T cells. In the periphery LECs bidirectionally communicate with T cells and suppress autoreactivity. In autoimmune disease models, meningeal T cells have been shown to interact with LECs but the role meningeal LECs play in maintaining T cell homeostasis requires more thorough investigation. The zebrafish is an experimentally and genetically tractable immune model that enables live imaging of immune cells with homologs for 80% of human immune genes. We have already demonstrated that meningeal blood and lymphatic vessels and meningeal immune cell populations can all be live imaged through the thin, transparent skull of the adult zebrafish using available cell-type specific transgenics, and cerebrovascular injury has been modeled in zebrafish. We are now using this superb model to study how LECs influence T cell identity and migration in the meninges. scRNAseq of zebrafish meninges reveals multiple T cell clusters, many of which have a naïve, patrolling phenotype at steady state. Angiographic imaging suggests intracranial lymphatics are connected to the olfactory system, dorsal lymphatic vessels, as well as to the kidney marrow. We plan to investigate how LECs influence T cell trafficking and phenotypes after cerebrovascular injury and molecular mechanisms of crosstalk between LECs and T cells, with the goal of leveraging this understanding to help develop new treatments for chronic neuroinflammatory disease.

Keyword: *T cells, Lymphatic endothelial cells, zebrafish*

#183 Genome-wide CRISPR Screen Reveals Essential Modules Regulating T Cell Migration In Experimental Autoimmune Encephalomyelitis

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Autoreactive CD4+ T cells are known to play a significant role in the development of multiple sclerosis (MS) by being the first immune cells to invade the central nervous system (CNS). In the experimental autoimmune encephalomyelitis (EAE) animal model, the transfer of in vitro activated encephalitogenic CD4+ T cells into naïve Lewis rats induces the disease, highlighting the importance of T cell infiltration into the CNS.

Consequently, identifying factors involved in T cell entry into the CNS presents a promising avenue for potential treatment strategies. While previous studies have primarily focused on adhesion molecules involved in T cell trafficking, the contribution of other components in facilitating T cell entry into the CNS remains unclear.

To gain insights into molecules essential for T cell migration during the initial phase of EAE, we conducted an unbiased whole-genome in vivo CRISPR screening. Through this screening, we identified 18 genes whose knockout prevented T cell entry into the CNS, and 5 genes where knockout resulted in enhanced migration. Further validation studies allowed us to categorize these genes into three functional key modules.
The first module, the adhesion module, consists of Integrin alpha 4 and its chaperone Hsp90b1. Deletion of these genes reduced the ability of T cells to attach to endothelial cells at the blood-brain barrier, impairing their migration into the CNS. The second module, the chemotaxis module, involves the chemokine receptor Cxcr3, its intracellular binding partner Gnai2, and the transcription factor Tbx21. Alterations in these genes disrupted the chemoattraction process, resulting in reduced T cell migration into the CNS. The third module, known as the egress module, revolves around the kinase Grk2. In vivo microscopy in the spinal cord leptomeninges revealed that GRK2-deficient T cells were capable of adhering to endothelial cells but failed to complete the diapedesis process. Further in vivo experiments demonstrated that this effect was mediated through the internalization of S1PR1, a signaling molecule involved in cell migration.

Collectively, our study defines essential modules involved in T cell migration during the initial phase of EAE, potentially paving the way for the identification of new therapeutic targets to regulate T cell infiltration into the CNS.

Keyword: *autoimmunity, multiple sclerosis, experimental autoimmune encephalomyelitis, T cell migration, CRISPR gene editing*

#201 Amyloid beta (Aß)-related T cell infiltration and crosstalk between microglia and T cell subsets in the App23 mouse model

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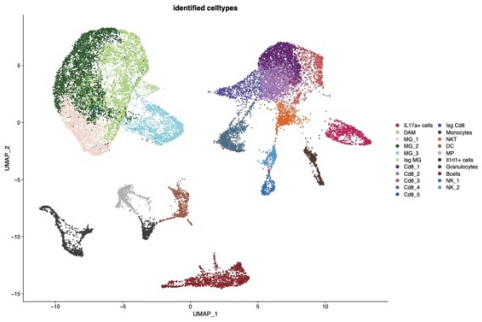
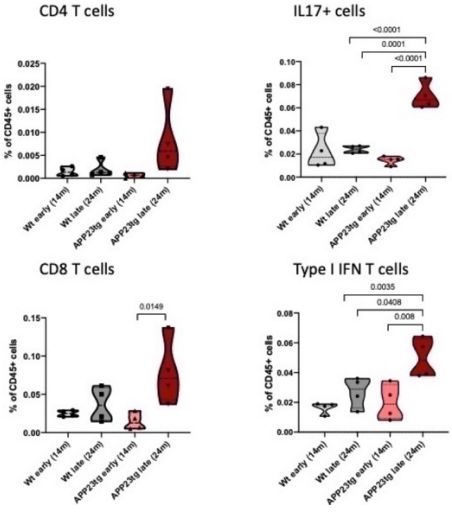
For decades, neuroinflammation has been recognized as a contributing factor in amyloid beta (Aβ)-related pathologies such as Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). While the role of the innate immune system has been extensively studied over the last years, the inflammatory contribution of adaptive immune cells, particularly T cells, remains less well understood. To address the role of central nervous system (CNS)-infiltrating T cells in cerebral Amyloid beta (Aβ)-related disease, we conducted single-cell RNA sequencing at an early (14 months) and late time point (24 months) of disease in the App23 transgenic mouse model, which carries the pathogenic Swedish mutation leading to a 7-fold overexpression of human amyloid-precursor-protein (APP), making it ideally suited to study the effect of extracellularly accumulated Aβ-plaques on neuroinflammation.

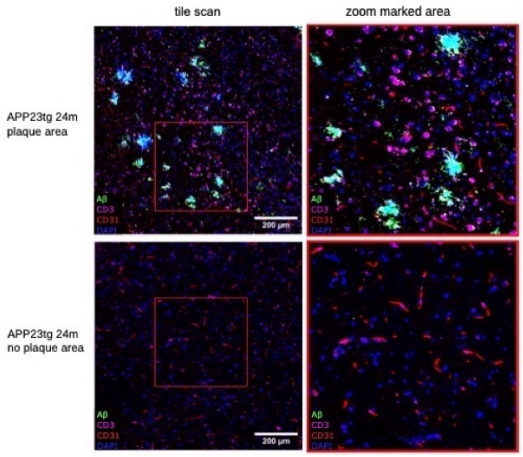
Using single-cell sequencing of CNS-infiltrating CD45+ cells we observed a significant infiltration of T cells in the brain over time with the number

of T cells correlating with plaque load and thus disease severity. Moreover, histological analysis revealed that T cells are more attracted to parenchymal as compared to vascular plaques, indicating stronger inflammatory homing cues surrounding parenchymal lesions. In addition, we found that the transition from early to late disease is accompanied by a shift in the immune response, from a microglia-dominated phenotype in early disease towards a more T cell-dominated phenotype in late disease. Finally, by examination of the transcriptomic landscape of T cells, we successfully identified two distinct subsets that exhibit a pronounced upregulation in late-stage disease. One subset is characterized by an interleukin (IL17) signature, the other one by a Type I Interferon (IFN) signature, both of which are widely acknowledged to be linked to neuroinflammation, loss of synapses, and disruption of the blood-brain barrier, suggesting that these subsets play a significant role in driving disease progression.

Collectively, our findings underscore the importance of T cells in the pathogenesis of Aβ-related diseases and highlight the role of IL17 and Type I IFN signaling in T cells during late-stage disease, simulating neuroinflammatory processes and disease progression. These results provide valuable insights into the adaptive immune response in Aβ-related pathologies and may open new avenues for therapeutic interventions targeting T cell-mediated neuroinflammation in AD and CAA.







Keyword: *Alzheimer's disease, amyloid beta, T cells, IL17 & Type I Interferon, single-cell sequencing*

#210 In depth analysis of CD4+ T cells in chronic neuroinflammation reveals a differential distribution of distinct subpopulations

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Introduction: Multiple sclerosis patients undergo a highly variable interindividual disease course. Understanding the pathogenic immune cell subsets at the initial diagnosis may be crucial for developing personalized treatments and defining therapeutic monitoring targets. Although recent single-cell studies have identified potential cell subsets in small cohorts of MS patients at different disease stages, pathogenic subsets driving the inflammation within the central nervous system (CNS) tissue remain unclear.
Objectives: Characterization of immune cell subsets relevant for the inflammatory pathogenesis in chronic experimental neuroinflammation and validation of markers in early MS

Methods: We utilized an animal model of experimental autoimmune encephalomyelitis (EAE) to analyze CD4+ T cells during acute and chronic phases in CNS tissue using single-cell transcriptome analysis. Subsequently, we designed a marker panel (n=27) for multi-dimensional spectral flow cytometry, incorporating findings from published single-cell datasets and the animal study. We recruited patients (n=22) with newly diagnosed MS and a control group with suspected idiopathic intracranial hypertension (IIH). Routine cerebral spinal fluid (CSF) samples were analyzed using flow cytometry (Cytek Aurora).

Results: In the EAE model, we identified five distinct subsets of CD4+ T cells in CNS tissue, three of which are previously uncharacterized, namely innate-like, tissue resident memory, and lymphoid tissue-like CD4 T cells. During the chronic phase, we observed a change in cluster distribution, with an expansion of tissue-resident regulatory CD4+ T cells and a shift towards a cytotoxic phenotype in the effector subset. Analysis of human CSF cell analysis demonstrated elevated markers between MS and IIH, with varying upregulation patterns of the acute and chronic phases of EAE. Notably, early-phase MS patients could be separated in two groups according to the distribution of effector and memory-like CD4 subsets in the CSF.

Conclusion: Translating markers from in-depth phenotyping of CD4+ T cells during acute and chronic EAE to human CSF reveals a differential distribution of specific CD4+ T cell subsets in early MS patients. While further prospective evaluation of these patients is necessary to determine the clinical significance of these findings, we generally show that spectral flow cytometry enables cost-effective and patient-specific deep phenotyping of immune cells in large cohorts.

#222 Extensive T cell profiling following SARS-CoV-2 mRNA vaccination in multiple sclerosis patients treated with DMT's

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Introduction: Messenger ribonucleic acid (mRNA) vaccines played a crucial role in the fight against the SARS-CoV-2 pandemic. This single centre prospective study aimed to evaluate SARS-CoV-2 specific T cell response after mRNA vaccination in patients with multiple sclerosis (PwMS) treated

with disease modifying therapies (DMT's) and to compare T with B cell response.

Methods: T cell immunity of 47 PwMS with or without disease modifying treatment on natalizumab, alemtuzumab (control group) versus ocrelizumab or fingolimod was evaluated. Peripheral blood mononuclear cells at different timepoints (baseline, 1 month, 2 months, 6 months after vaccination) were stimulated with SARS-CoV-2 specific antigens S, S1, M and N. Changes of intracellular cytokines as well as expression of surface activation and differentiation markers on CD4+ and CD8+ T cells were assessed by FACS analysis. B cell response was defined by detection of SARS-CoV-2 specific antibody levels.

Results: T cell profiling could prove the induction of SARS-CoV-2 specific T cell immunity in all patients' groups to various degrees. CD4+ high T cells increased after vaccination ($p < 0.001$, Figure 1A) whereas CD4+ low T cells presented a decreasing trend. CD8+ T cells number showed no significant changes after vaccination. CD4+ cytokine expression levels after vaccination were more effective for CD154 compared to IFN-gamma, TNF-alpha, and IL-2. All T cell subsets showed an increasing trend but only CD4+CD154+ T cells increased significantly one ($p < 0.001$), two ($p < 0.01$) and six ($p < 0.05$) months after vaccination (Figure 1B). Interestingly, there were higher levels of CD4+CD154+ T cells in PwMS with negative antibody response compared to PwMS with positive antibody response ($p < 0.05$). CD8+ cytokine expression levels showed no significant differences. CD4+CD38-HLADR+ T cells decreased six months after vaccination ($p < 0.05$).

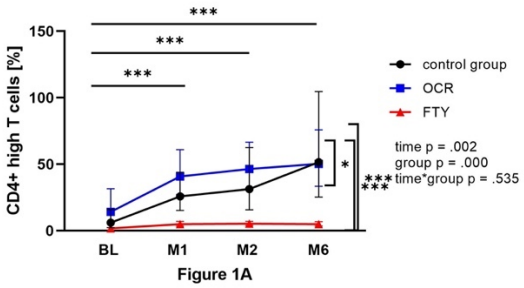

Figure 1A

Relative cell count of CD4+ high T cells. Analysis was performed before vaccination (BL), 1 month after vaccination (M1), 2 months after vaccination (M2) and 6 months after vaccination (M6). Mean values with 95% confidence interval are presented. Asterisks indicate a statistically significant difference between selected time points or groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

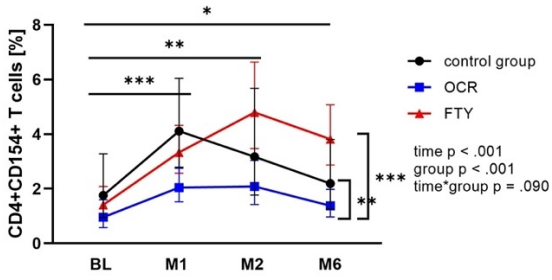

Figure 1B

Relative cell count of CD4+ CD154+ T cells. Analysis was performed before vaccination (BL), 1 month after vaccination (M1), 2 months after vaccination (M2) and 6 months after vaccination (M6). Mean values with 95% confidence interval are presented. Asterisks indicate a statistically significant difference between selected time points or groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Conclusion: Data indicate that mRNA vaccination can efficiently generate SARS-CoV-2 specific cellular immune responses in various treatment groups in PwMS. Moreover, cellular immune response might play a crucial role in PwMS when there is no humoral immune response detectable.

Keyword: *SARS-CoV-2, mRNA vaccines, T cell response, multiple sclerosis*

#224 Modulation of T cells by Vitamin D supplementation in MS

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Multiple sclerosis (MS) is an autoimmune chronic inflammatory disease of the central nervous system (CNS). T cells play an important role, and their migration to the CNS is key to MS pathogenesis. Vitamin D (VitD) has shown a significant correlation with increased MS risk and severity, leading to suggestions of VitD supplementation as a potential treatment for MS. However, the effectiveness of vitamin D supplementation in improving MS remains a topic of debate. We previously observed a modulation of adhesion molecules on the surface of activated CD4+ T cells by calcitriol (the active form of VitD). Our aim was to determine the impact of VitD on the phenotype of T cells in MS. In this study, we analyzed the phenotype of T cells from patients with Clinically Isolated Syndrome (CIS) before and after 3 months of placebo (n=6) or supplementation with a high dose of cholecalciferol (100,000 IU every 14 days, n=6) (D-lay MS clinical trial - NCT01817166), allowing the analysis of the effect of VitD in patients in the absence of any other immunomodulatory treatments. The data were analyzed using both manual gating and unsupervised analysis. Unsupervised analyses showed a significant increase in the proportion of effector T cells in the vitamin D-treated group. Phenotypic changes could also be observed, notably in adhesion molecules, between the two groups. These preliminary data suggest a modulatory effect of Vitamin D on the phenotype and proportion of T cells in patients with CIS. Longer follow-up of these patients will reveal whether these changes are related to clinical benefits.

This work is funded by the ARSEP foundation and the Agence Nationale de la Recherche (ANR - 19 - CE14 - 0043).

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Keyword: *T cells, Vitamin D, Migration, Multiple sclerosis (MS), Clinically Isolated Syndrome (CIS)*

#226 CD8+ T cell generated CD4+ T cells are important for the pathogenicity and infiltration of CD8+ T cells to CNS in an adoptive transfer EAE model.

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Both $CD4^+$ and $CD8^+$ T cells play critical roles in the immunopathogenesis of MS. 1C6 T cell receptor transgenic (TcR-Tg) mice on the nonobese diabetic (NOD) background have a $MOG_{[35-55]}$-specific, MHC class II-restricted, TcR that selects for both $CD4^+$ and $CD8^+$ T cells. We recently demonstrated that in vitro-differentiated 1C6 CD4 Th1 and Th17 cells can induce a progressive form of experimental autoimmune encephalomyelitis (EAE) upon adoptive transfer to NOD. Scid recipient mice. In this study, we assessed whether the same is true for 1C6 $CD8^+$ T

cells. 1C6 CD8 T cells differentiated and activated in response to plate bound anti-CD3/CD28 stimulus and also to the MOG_{35-55}-antigen+APCs. Upon adoptive transfer, 1C6 Tc1 and Tc17 cells ($5x10^6$ per recipient) induced progressive EAE. However, a disease of increased severity and a higher number of $CD8^+$ T cells was seen in the CNS upon co-transfer of Th1+Tc1 ($2.5x10^6$ of each per recipient). Intriguingly, ex vivo analysis of the spleens and CNS of Tc1-alone or Tc17-alone recipients revealed the presence of $CD4^+$ T cells. This was observed even in mice receiving $CD8^+$ T cells that were purified by high-speed cell sorting upon initial isolation from 1C6 mice; after in vitro culture but just prior to injection; and also at both time points. In vivo blockade of CD4 reduced not only the presence of $CD4^+$ T cells in the CNS of Tc17 alone recipients but also the presence of $CD8^+$ T cells; the frequency of splenic $CD8^+$ T cells was either increased or not affected. This suggests that CD4/CD8 cooperation is required for the infiltration of the target organ by the latter. Together, our data indicate that the presence of both $CD4^+$ and $CD8^+$ T cells specific for the same CNS antigen is required for optimal $CD8^+$ T cell pathogenicity in an adoptive transfer EAE model.

#233 CD20+ T cells in early multiple sclerosis

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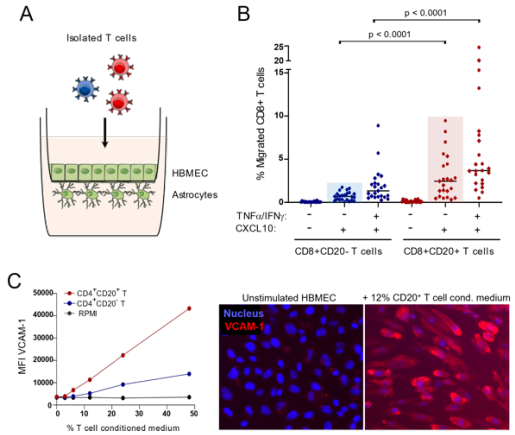
Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system (CNS). Within the last years it has become clear that early interventions to suppress the immune system improves the course of the disease. However, one of the difficulties in optimizing treatment is understanding how exactly the disease initiates; i.e. identifying the first reactions of the immune system. Using our in vitro human blood-brain-barrier (BBB) model of CNS-transmigration of immune cells based on a co-culture of primary human astrocytes and human brain microvascular endothelial cells (HBMEC; figure A), showed that T cells expressing the molecule CD20, $CD20^+$ T cells, in contrast to $CD20^-$ T cells have the ability to cross a non-inflammed BBB (figure B). Analyzing cerebrospinal fluid (CSF) of healthy donors identified $CD20^+$ T cells, indicating a role of $CD20^+$ T cells in CNS immunosurveillance and likely explains why $CD20^+$ T cells have the ability to cross the non-inflammed BBB as observed in our in vitro study.

In response to inflammation in the CNS, endothelial cells of the BBB upregulate adhesion molecules and astrocytes produce chemokines to attract peripheral immune cells to the CNS. In this study, we found that HBMEC grown in $CD20^+$ T cell conditioned medium upregulates the adhesion molecule VCAM-1 and ICAM-1 (figure C), and that astrocytes strongly increased their production of the chemokines CXCL9, CXCL10, CXCL11 and CCL5. This suggests that $CD20^+$ T cells migrated to the CNS in early MS produce proinflammatory molecules facilitating or amplifying the recruitment of peripheral immune cells.

Altogether, our data indicate a role of $CD20^+$ T cells in the very early steps of MS

immunopathogenesis.



Keyword: *CD20+ T cells, Multiple Sclerosis, Blood-brain-barrier*

#255 T cell phenotypes in Rasmussen Encephalitis

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Rasmussen encephalitis (RE) is a rare disease that mainly affects young children. Inflammation, neuronal loss, the formation of microglia nodules and astrogliosis are histopathological hallmarks. Remarkably, the seizure activity and inflammation occurs in a single hemisphere. Theories about viral or autoimmune origin have been suggested, yet the underlying cause remains enigmatic. It is undisputed that cytotoxic T cells are the driving force.

Here we hypothesized that infiltrating alpha beta (AB) and gamma delta (GD) T cells change their phenotype from effector T cells to T resident memory (TRM) cells after disease initiation. Therefore, we quantified T cell subpopulations in formalin-fixed paraffin embedded (FFPE) samples of 11 RE patients using multiplex spectral analysis. Additionally, we performed spatial transcriptomic on 2 FFPE samples of RE patients.

Our preliminary data with markers CD103, CD69 and CD49a reveal that a subpopulation of infiltrating T cells show a TRM phenotype. The relative proportion of TRMs ($CD103^+CD3^+$) among all T cells ($CD3^+$) is significantly higher in advanced stages with neuronal loss. Furthermore, our analysis shows that the TRM phenotype not only is found in T cells with the AB T cell receptor (TCR) but also in T cells with the GD TCR: Up to 26% of all $CD3^+$ T cells express the GD T cell receptor (TCR). Moreover, GD T cells expressing Granzyme B (GrB) were also seen attached to neurons.

Taken together our data provide a first insight into the composition of T cell infiltrates in RE, a prime example for a T cell mediated encephalitis. Our findings suggest that, like in tumors, the loss of a pathogenic or autoimmune antigen drives the effector T cells into the TRM phenotype. The presence of GrB+ GD T cells attached to neurons and the presence of these cells with a TRM phenotype suggests that these cells, despite acting MHC independent, are antigenic in nature. Currently, we are actively investigating additional markers for TRMs as well as immune checkpoint pathways. The combination of histological findings with the data obtained by spatial transcriptomics, will provide further detailed insight into the different T cell phenotypes and their role in inflammatory brain diseases.

#303 Microbiota induce activation of encephalitogenic T cells and following T cell infiltration into the central nervous system

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**Equal contribution*

The human immune system harbors a diverse repertoire of encephalitogenic T cells that persist even in healthy individuals. However, their disease-causing potential remains dormant until a yet unknown trigger activates them, leading to their infiltration into the central nervous system (CNS). Once within the CNS, they induce local inflammation, ultimately triggering diseases such as multiple sclerosis (MS). Despite extensive research efforts, the precise mechanism of this trigger remains elusive. However, emerging studies strongly indicate a significant contribution of the microbiota in this intricate process. In pursuit of understanding the trigger within gut-associated lymphoid tissues (GALT), we utilized state-of-the-art intravital imaging techniques. Moreover, we examined the destiny of activated T cells within this specialized environment of the GALT.

Intravital imaging demonstrated that MOG-specific CD4$^+$ T cells exhibited higher levels of calcium activity in the lamina propria (LP) of the ilium compared to polyclonal T cells, indicating the activation of the MOG-specific CD4$^+$ T cell population. Strikingly, this enhanced calcium signalling was absent in MOG-specific CD4$^+$ T cells residing in Peyer's patch, underscoring the tissue-specific nature of the activation process. Intriguingly, analogous observations were made with other transgenic T cell populations, including OVA-specific CD4$^+$ T cells and LCMV-specific CD4$^+$ T cells, which also exhibited elevated calcium signalling within the LP. Remarkably, the administration of anti-MHC class II antibody resulted in a discernible reduction in calcium activity specifically within MOG-specific CD4$^+$ T cells, implicating the involvement of MHC class II molecules in this activation cascade. Furthermore, the absence of elevated calcium signalling in MOG-specific CD4$^+$ T cells imaged within the LP of germ-free mice lends further support to the notion that both the microbiota and MHC class II molecules play crucial roles in orchestrating the activation process.

Transcriptome analysis of T cells revealed an intriguing pattern in GALT-emigrating MOG-specific CD4$^+$ T cells, as they exhibited a marked upregulation of Th17-related genes, notably IL-22 and IL-23R, along with migration-related genes including IL1R and CCR6, when compared to their counterparts in the spleen. These findings suggest that T cells stimulated within GALT acquire a migratory Th17 phenotype, which has emerged as a pivotal player in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an established animal model for MS. To further investigate the migratory potential of these cells, we conducted an in vivo migration assay employing transgenic mice expressing a photoconvertible protein. Remarkably, our results showed the trafficking of MOG-specific CD4$^+$ T cells from small intestine to CNS in presymptomatic mice.

Taken together, our findings provide compelling evidence of the microbiota's profound influence on the induction of the Th17 phenotype and subsequent infiltration of these cells into CNS,

serving as a crucial initial event in the pathogenesis of EAE.

Keyword: *CD4+ T cells, Intravital imaging, microbiota, calcium signaling*

#307 Evaluating the pathogenicity of T cells in Alzheimer's Disease and other Tauopathies

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#Contributed equally to this work

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline and memory loss. It is defined by the accumulation of amyloid-beta peptides in extracellular deposits, intraneuronal fibrillar aggregates of hyperphosphorylated tau proteins and chronic innate neuroinflammation mediated by microglia and astrocytes. Besides neuroinflammation, dysregulation of peripheral immunity and peripheral-central immune crosstalk has emerged as a key feature of AD. Recent findings highlight the critical role of T-cell immunity in the pathophysiology of AD and other Tauopathies. We previously evidenced that tau pathology is associated with T-celldependent detrimental processes that contribute to promote disease progression. However, the characteristics, functionality and antigen specificity of such detrimental T cell responses remain poorly defined. As meningeal lymphatics critically contribute to brain immunosurveillance by draining macromolecules from the central nervous system (CNS) towards cervical lymph nodes (cLNs), we hypothesize that pathological tau proteins may drain into the cLNs, resulting in the priming of tau-specific T-cell responses, which subsequently infiltrate the brain and promote disease progression.

To investigate the pathogenic potential of cLN-derived T cells in Tauopathies, we conducted adoptive transfer studies in the THY-Tau22 mouse model of AD-like tau pathology. We evaluated the impact of cLN-derived T cells on cognitive functions, central neuroinflammation and tau pathology. We also assessed the reactivity of cLN-derived T cells to pathological tau proteins by in vitro antigen restimulation assays.
Our preliminary results indicate that along tau pathology, cLN-derived T cells i) modulate the activation profiles of microglia and astrocytes, ii) selectively enhance the infiltration of CD3+ T cells in the brain parenchyma and hippocampus, iii) accelerate the onset of cognitive deficits in young diseased mice, and iv) slightly alter the extent of tau deposition.

Our findings support that accumulation of pathological tau proteins selectively triggers pathogenic T-cell responses in brain-draining cLNs, which subsequently infiltrate the brain and promote tau-related neuroinflammation and cognitive decline. Ongoing studies aim at further deciphering the role of CD4+ and CD8+ T cell subsets in disease progression and the functionality and antigen specificity of CNS-associated T cells in response to tau pathology.

Keyword: *Alzheimer's Disease, Tau pathology, Neuroinflammation, Neurodegeneration, T-cell immunity*

#312 Unravelling adaptive immune response in patients with ischemic stroke using single-cell RNA-sequencing

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The global burden of ischemic stroke (IS) is high and set to rise over the next decades. Experimental stroke models suggest an important neuroprotective role of anti-inflammatory regulatory T cells (Tregs) in enhancing neuronal recovery after ischemia. To confirm these findings in patients after stroke, we quantified components of adaptive immune response in peripheral blood (days 0, 3, 7, 10, 14, 17, 21, 24 and 28), cervical lymph nodes (cLNs) of carotid endarterectomy patients (CE) and brain of patients (post-mortem) after IS caused by large cerebral blood vessel occlusion and compared results with matched controls. In peripheral blood, we observed increased levels of T cells in the IS group throughout the nine time points compared to the controls. Levels of Tregs in IS patients were slightly lower than control levels at admission and decreased until day 7. However, from day 7 until day 10 an increase in the percentage (p-value <0.05) of Tregs was observed, which were maintained at levels comparable to the controls until day 28. In IS patients, preliminary unsupervised clustering of bulk transcriptomes on sorted Tregs indicates phenotypical differences between the early (d0-14) and late time (d17-28) points. Our data show that the expression of anti-inflammatory markers such as LIF and IL10 peaks between day 14 and d17. Moreover, peripheral Tregs show high expression of the proliferation marker Ki-67 throughout all the time points. Late Tregs (d17-28) show higher expression of Tregs markers associated with immunosuppressive functions such as FOXP3, STAT5 and CD69. Cervical cLNs biopsies confirmed these findings showing a higher presence of T cells (p-value <0.0001) and Tregs (p-value =0.081) post-stroke using flow cytometry analyses and immunofluorescence compared to control autopsies. Moreover, single-cell RNA-sequencing analysis coupled with TCR profiling has indicated higher TCR diversity in the cLNs (average paired clonotype diversity = 1700) compared to matched PBMCs (average paired clonotype diversity = 231). Flow cytometry ratio of IS autopsy samples showed a 1:1 ratio of Tregs over T helper, matching previous findings of brain regulatory T cells in middle cerebral artery occlusion mouse model. Our results show the relative increase in and migration of regulatory T cells in patients after ischemic stroke and confirm the important role of the cLNs nodes in this process. Our findings in patients align with previous experimental studies regarding Tregs population dynamics. In addition, we uncovered unknown molecular features of Tregs. Altogether, these results may be useful for developing new Tregs-based therapeutic strategies for IS.

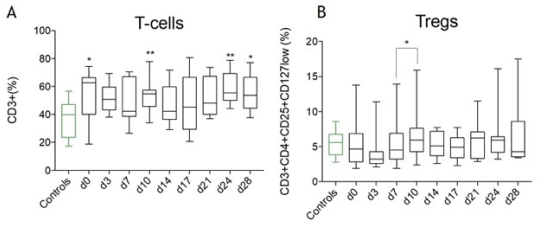

Figure 1 – Characterization of the adaptive immune response in peripheral blood after IS by flow cytometry. Analysis of the percentage of T cells and Tregs is performed. A single time-point represents controls (green bars, n=10), and stroke patients (black bars, n=10) are presented at nine time-points both by percent positivity of (A) T-cell lymphocytes and (B) Regulatory T-cells (Tregs). Data is presented as mean ± standard deviation (SD). Statistical analysis was

performed using the Mann-Whitney test between individual time-points and controls and significant p-values indicated as * ($P < 0.05$), ** ($P < 0.05$), *** ($P < 0.0005$).

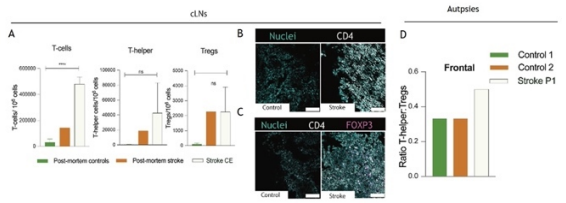

Figure 2 - Characterization of the adaptive immune response in cervical lymph nodes of IS patients who underwent carotid endarterectomy and autopsy samples by flow cytometry and immunofluorescence. (A) Cervical lymph nodes were analyzed by flow cytometry regarding prevalence of T-cells, T-helper and Tregs per 10^6 cells and compared with postmortem cervical lymph nodes of controls (n=2) and ischemic stroke autopsy (n=1). Statistical analysis was performed using the Mann-Whitney test and significant p-values indicated as * ($P < 0.05$), ** ($P < 0.05$), *** ($P < 0.0005$). (B) Immunofluorescence staining for CD4 (gray) is presented for carotid endarterectomy after stroke and controls (C) Immunofluorescence staining for CD4 (gray) with FOXP3 (pink) is presented for carotid endarterectomy after stroke and for control cervical lymph nodes identifying Tregs. In both immunofluorescence panels cell nuclei is stained with propridium iodide (PI, green) (Scale bars, 50µm). (D) bar plot representing flow cytometry ratio of Tregs in relation to regular T-helper from the total CD4+ fraction on single samples from frontal one stroke autopsy and two controls.

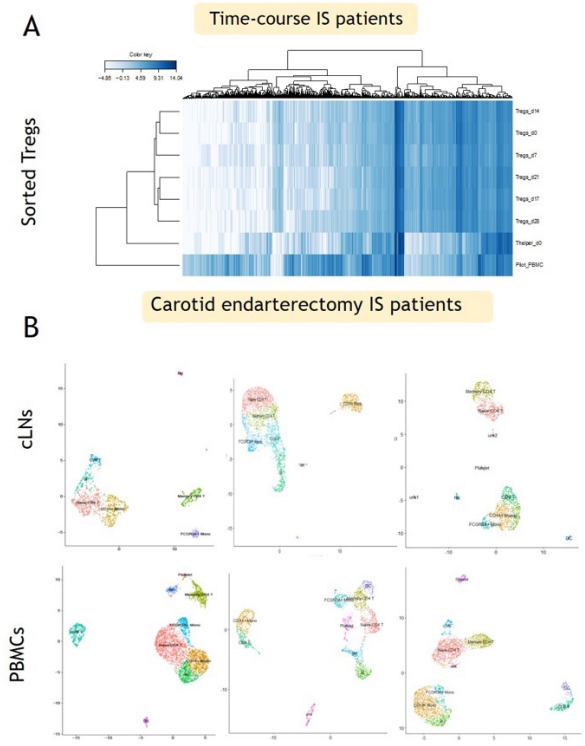

Figure 3 - Heatmap of different clusters of DE genes Tregs over time (d0,7, 14, 17, 21, 28), Thelper (d0) and PBMCs were included as outgroups for initial bulk RNA-seq analysis. The colors in the heatmap indicate high (blue) or low (white) expression across the sample set (B) UMAP dimensionality reduction based on global gene expression of all CD45+ cells from cLNs and PBMCs of IS patients that underwent carotid endarterectomy (n=3).

Keyword: *Ischemic stroke, Regulatory T cells, Tregs, Single-cell RNA-sequencing*

#329 Simultaneous single-cell RNA and ATAC sequencing of peripheral lymphocytes of MS-discordant monozygotic twins

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Introduction:

Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system caused by an interplay of genetic susceptibility and environmental risk factors. Certain HLA associations and single nucleotide polymorphisms are known risk factors for MS development. Despite that, identical twins only have a 17% age-adjusted risk of developing MS concordantly. This suggests that dysregulation of gene loci may be involved in MS pathogenesis. To investigate the functional relevance of chromatin accessibility in immune cells with an identical genetic background but discordant disease phenotypes, we applied MULTIOME to a unique cohort of MS-discordant monozygotic twins.

Methods:

For this study, we selected 5 monozygotic twin pairs discordant for MS from the MS TWIN STUDY (all RRMS, untreated, x̄ age: 33). To examine gene expression and gene regulatory mechanisms, we employed a MULTIOME approach, combining single-cell RNA sequencing and transposase-accessible chromatin sequencing (ATAC-seq) in peripheral blood mononuclear cells. Bioinformatical pipelines utilizing various enrichment tools were used, we performed peak calling via MACS2 (Model-based Analysis of ChIP-seq) and analyzed DNA accessibility using latent semantic indexing (LSI).

Results:

We successfully generated RNA and ATAC libraries and employed bioinformatical co-embedding, to identify 8 distinct cell populations. These were annotated as natural killer (NK) cells, mucosal-associated invariant T (MAIT) cells, CD4+ and CD8+ T cells, each exhibiting distinct differences in gene expression and chromatin accessibility (UMAP Fig. 2). Further, we identified enhancer-promoter connections and their regulatory effects on different genes using the scREG package. Our findings demonstrate quantitative expression of cell-type-specific markers, such as NKG7 in cytotoxic T cells, as well as interchromatin links, and allow to construct subpopulation-specific cis-regulatory networks (Fig. 3).

Conclusions:

This pilot study established an innovative MULTIOME approach in a unique twin setting, offering a preliminary dataset to explore chromatin accessibility modifications and dynamic chromatin remodeling in immune dysregulation. Despite the high costs and methodological challenges, MULTIOME has shown promise as a valuable tool. Our refined bioinformatic analysis, with additional samples including cerebral spinal fluid, is expected to provide valuable insights into the autoimmune pathogenesis of MS.

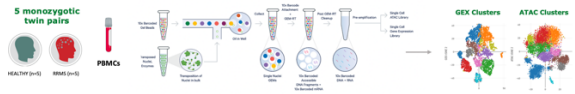

Figure 1: Summary of the cohort and processing of PBMCs from the MS TWIN STUDY. Icons were created with BioRender. Partial source: 10xgenomics.

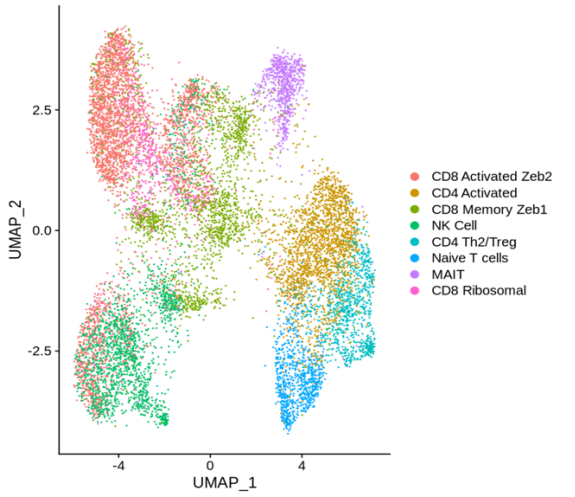

Figure 2: UMAP of the different cell clusters.

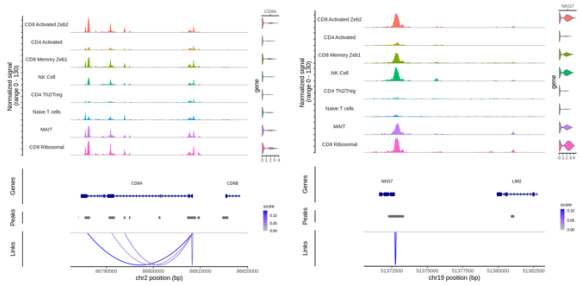

Figure 3: Plot shows the correlation of genes, peaks and links for the markers CD8A and NKG7 (marker for cytotoxic CD8+ cells). Quantitative gene expression is shown, with the associated enhancers-promoter region. The exact chromatin site, as well as the connection between promoter and enhancer, is displayed.

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Keyword: *Multiple sclerosis, neuroimmunology, ATAC sequencing, twins, MULTIOME*

#332 Preclinical evaluation of a Treg-targeting immunomodulatory treatment in a mouse model of Alzheimer-like tau pathology

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[#]Contributed equally to this work

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and cognitive functions. Its main neuropathological hallmarks are the aggregation of Abeta amyloid peptides in the form of extracellular amyloid plaques, intraneuronal aggregation of abnormally phosphorylated Tau proteins in the form of neurofibrillary tangles, and chronic innate neuroinflammation mediated by microglia and astrocytes. Besides neuroinflammation, increasing evidence also highlight an instrumental role of peripheral immunity and peripheral-central immune

crosstalk in AD pathophysiology. Our previous studies in a mouse model of AD-like amyloid pathology evidenced a beneficial role of regulatory T cells (Tregs), which modulate the rate of disease progression and the onset of cognitive deficits, at least partially by controlling the development of detrimental microglial responses in favor of beneficial innate neuroinflammation. Our recent data further supported that Tregs also modulate and fine-tune the balance of reactive astrocyte subtypes in AD-like amyloid pathology, by dampening C3-positive astrocytes. In parallel, in a mouse model of AD-like Tauopathy we previously evidenced that Tau pathology is associated with detrimental T-cell-mediated processes that contribute to promote Tau-related detrimental neuroinflammation and cognitive deficits. Considering the unique capacity of Tregs to inhibit both $CD4^+$ and $CD8^+$ T cell responses, our data raise the hypothesis that amplifying Tregs may allow controlling Tau-driven T-cell-mediated detrimental processes in the course of AD and other Tauopathies. We thus evaluated preclinically the impact on disease progression of an optimized IL-2-based Treg-targeting immunomodulatory treatment in the THY-Tau22 mouse model of Tauopathy. Our data support that such treatment aimed at selectively amplifying Tregs i) restores cognitive functions, ii) modulates functional profiles of astrocytes and microglia, including markers of Disease-Associated Microglia (DAM) and reactive astrocyte subsets, and iii) may partly alter pathological Tau protein deposition. Our study supports the therapeutic potential in Tauopathies of Treg-targeting immunomodulatory approaches.

#348 Helicobacter pylori induces an auto-reactive immune phenotype in PINK1 deficient mice that correlates with motor dysfunction.

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Parkinson's disease (PD) is a chronic neurodegenerative disorder. Several genetic predispositions, including the PTEN-induced kinase 1 (PINK1) mutation, have been implicated in early onset family cases. Besides the loss of dopaminergic neurons in the brain, PD patients have a unique immune phenotype that includes increased inflammation, blood serum, brain levels of proinflammatory cytokines, brain infiltration with cytotoxic CD8 T cells, and loss of regulatory T cells (Treg) and their anti-inflammatory phenotype.

The role of the gut microbiota and gastrointestinal infections are increasingly recognized as a cofactor in PD. One of pathogens associated with the risk of PD is Helicobacter pylori. The prevalence of this Gram-negative bacteria in PD patients is higher than in the general population and its eradication in PD patients improves motor function.

Loss of the PD-associated PINK1 alters induced Treg function in vitro. We previously have shown that in PINK1 knock-out (KO) mice, gut infection with Gram-negative bacteria, Citrobacter rodentium, induces mitochondrial antigen presentation (MitAP) to the CD8 T cells that later infiltrate the brain. In this model, PINK1 KO mice develop a Parkinson-like L-DOPA-responsive motor phenotype after four C. rodentium infections.

Here we aimed to scrutinize potential role of the H. pylori infection in the induction of motor and immune phenotypes in PINK1 KO mice.

In addition to standard behavioural testing, at 2- and 6-months post-infection we performed a multiplex cytokine analysis of gastric homogenates and spectral flow cytometry of gastric lamina propria, mesenteric lymph nodes, blood, spleen, and brain infiltrating immunocytes.

We show that infection with H. pylori causes an immune phenotype that remains 6 months post-infection. This phenotype, though present in some WT infected mice, is higher in PINK1 KOs and similarly to PD patients includes a decrease in Treg proportion, FoxP3 downregulation, Th2 CD4 T cell loss, as well as increase of mitochondrial antigen-specific CD8 T cells. Moreover, the immune phenotype in the H. pylori infected PINK1 KO mice correlates with motor dysfunction and CD8 T cell brain infiltration with no such association seen in the WT littermate controls.

These results provide insight to the gut-immunity-brain axis in the pathogenesis of Parkinson's disease, and further investigate the role of Gram-negative bacteria in the establishment of immune tolerance-autoreactivity balance.

Keyword: *Parkinson's disease, Helicobacter pylori, Treg, Mitochondrial antigen presentation*

#354 Pioneer recruitment of CD4 CCR5+ cells via the blood-cerebrospinal fluid barrier promotes neuroinflammation during acute HIV infection.

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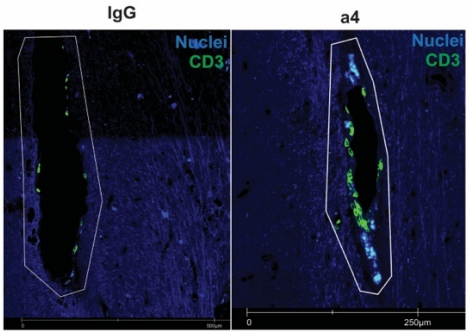
Effector CD4 T cell entry into the brain parenchyma can mediate subsequent activation of resident microglia and macrophages, a pivotal event in the initiation of neuroinflammation. Our previous study in a rhesus model of HIV has shown that CD4 T cell entry, particularly the CCR5+ subset, promotes both viral establishment and neuroinflammatory gene signatures within synapse-dense regions of the brain (Hawes and Elizaldi et al, JNI, 2022). These findings highlight the critical need to understand the mechanisms governing CD4 T cell entry into the brain. While considerable attention has been directed towards CD4 T cell entry across the blood-brain barrier (BBB), the blood-cerebrospinal fluid barrier (BCSFB), characterized by its high degree of permeability and abundance of CD4 T cells, has received comparatively less scrutiny. To investigate the distinct roles of the BCSFB and the BBB in CD4 T cell entry, we utilized a primatized monoclonal antibody to inhibit alpha 4 beta 1-mediated entry via the BBB barrier. This experimental approach was conducted during the acute phase of SIV infection in rhesus macaques; we treated animals with anti-Rh-alpha 4 [Rh-a4] (25mg/kg, n=4) or IgG isotype (n=4) before and during SIVmac251 infection. Rh-a4 resulted in complete alpha 4 receptor coverage across both the CSF and blood compartments, leading to profound lymphocytosis (approximately a 3-fold increase; $p<0.05$) prior to and during acute SIV infection (**See Figure 1**). Notably, alpha 4 blockade resulted in a significant increase in CSF CD4+ CCR5 frequencies in all treated animals, indicating either (1) increased retention of the subset in the CSF, (2) inhibition of trafficking from the CSF into the brain parenchyma across the BCSFB and/or (3) inhibition of trafficking from the CSF to the dural lymphatics. The assessment of blood-brain

barrier (BBB) integrity was assessed using albumin quotient, CSF total protein, and immunofluorescence evaluation of tight junction proteins and revealed that the BBB was not compromised. Intriguingly, animals treated with Rh-a4 exhibited an unexpected elevation in viral burden within the grey matter regions of the brain parenchyma compared to the IgG control group (**See Figure 2**). Assessment of soluble mediators demonstrated an increase in the Th1 chemokine IP-10 in both the CSF and Blood. Spatial transcriptomic analyses of the Hippocampus of anti-a4 treated pinpointed T cell accumulation within the blood vessels, with few T cells infiltrating the adjacent parenchyma (**See Figure 3**). Together our data strongly support the hypothesis that the initial recruitment of CCR5+ CD4 T cells through the BCSFB serves as a pioneer event, triggering neuroinflammation and subsequently promoting waves of T cell entry into the CNS.

Figure 1. Lymphocytosis is observed in the blood after Rh-a4 treatment and CCR5+ CD4+ T cells increase within the CSF



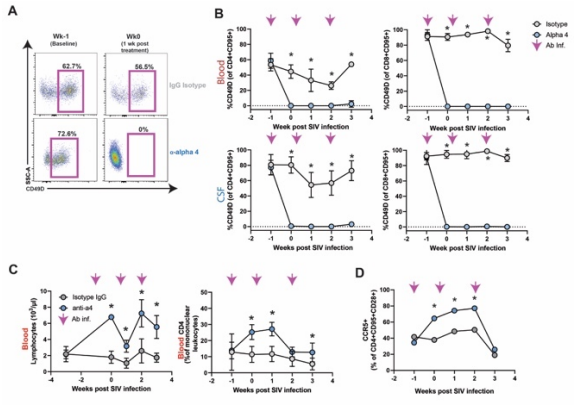


Figure 2. Increased viral burden in the grey matter of Rh-a4 treated rhesus macaques

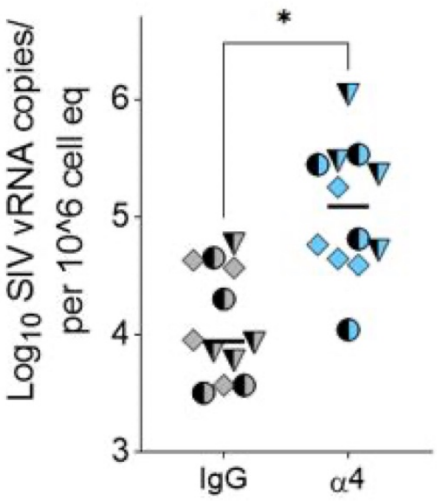


Figure 3. T cell accumulation in the blood vessels of Rh-a4 treated rhesus macaques

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CNS barriers

#32 Investigating the Role of the Dura Mater During Th17 Cell Induced Grey Matter Injury

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Multiple sclerosis (MS) is an inflammatory autoimmune disease of the brain and spinal cord whereby multiple immune cells collaborate to mediate white and grey matter demyelination as well as neurodegeneration. In MS, the normally immune-privileged leptomeninges lining the brain become populated with immune cell aggregates that are thought to contribute to cortical demyelination and progression of disability. While the brain and overlying leptomeninges are relatively immune-privileged at homeostasis, recent literature has identified the outermost meningeal layer, the dura, as a rich reservoir of immune cells. Recent literature has also shown evidence for "outward" movement of leptomeningeal $CD3^+$ cells into the dura as well as "inward" movement of calvaria bone marrow myeloid and B cells into the dura through diploic veins. This implies that there is cellular interchange between the BM, dura and leptomeningeal compartments. However, the relationship between leptomeningeal aggregates and the dura during MS and the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), has never been explored. Using a model of EAE that recapitulates aspects of MS cortical brain pathology associated with leptomeningeal inflammation, we have found that the frequency and number of $CD3^+$, $B220^{high}$ and $Ly6G^{high}$ cells increase in the dura and leptomeninges as EAE develops, with greater absolute numbers of these cells in the leptomeninges of EAE mice. Interestingly, two $B220^{intermediate}$ cell populations were observed in the naïve dura based on IL7R expression. Some $B220^{intermediate}IL7R^+$ cells were also Tdt^+, supporting recent literature showing the presence of developing pro- and pre- B cells in the naïve mouse dura. While $B220^{intermediate}IL7R^+$ cell populations decrease in the dura and leptomeninges during EAE, $B220^{intermediate}IL7R^-$ cells decrease in the dura while increasing in the leptomeninges. Future cell labelling and tracking experiments will determine if the increase in $B220^{intermediate}IL7R^-$ cells in the leptomeninges is due to trafficking from the dura. Such cell trafficking from the dura "inwards" to the leptomeninges has yet to be described, but this has not been tested in the context of underlying leptomeningeal inflammation such as that observed in A/T SJL/J EAE. Side by side comparison of scRNA sequencing of the dura and leptomeninges from naïve and EAE mice has revealed several pathways that are upregulated in EAE that may be driving immune cell movement between the two compartments. Some of these signaling molecules, such as Ccl8, have been validated by ELISA and shown to be increased in the dura. Flow cytometry and scRNA sequencing experiments have also revealed differences in stromal cell subsets between the dura and the leptomeninges that could explain the different roles of these tissues in inflammation. Ultimately, this research has the potential to discover driving factors in disease progression such as retention of pathologic or tolerogenic immune cells in the leptomeninges of the diseased brain. This knowledge can be used to design new therapeutic approaches that interfere with the accumulation of pathologic cells or supports the influx of tolerogenic cells.

Keyword: *meninges, CNS barriers, dura mater, leptomeninges, immune trafficking*

#34 ACE2 protein is increased in the parietal cortex of individuals with Alzheimer's disease

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[3]Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago USA

Background: The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a major cause of death, particularly in the elderly. The geriatric population, in which cognitive decline due to Alzheimer's disease (AD) is frequent, was disproportionately affected by the pandemic. In addition, central nervous system (CNS) manifestations have been reported in a significant subset of SARS-CoV-2 infected patients. **Methods:** Since the principal entry receptor utilized by SARS-COV-2 is Angiotensin-Converting Enzyme 2 (ACE2), we examined whether post-mortem ACE2 protein and mRNA levels were altered in parietal cortex samples from two different AD cohorts, totalling 142 cases. **Results:** Immunoblotting and RT-qPCR analysis revealed higher levels of soluble ACE2 protein and mRNA in persons with a neuropathological diagnosis of AD, compared to age-matched controls. Subjects with a clinical diagnosis of AD and cerebral amyloid angiopathy (CAA) also had higher levels of soluble ACE2. Brain levels of ACE2 were inversely correlated with antemortem cognitive scores. Immunostaining experiments showed that, even though cerebral ACE2 proteins were highly enriched in microvessels of mice compared to parenchymal cells, such was not the case in the human brain. The increase in soluble ACE2, which can be interpreted as a detachment of ACE2 from brain cell membranes, was strongly associated with pericyte loss. **Conclusion:** Our data suggest that a neuropathological diagnosis of AD and the presence of CAA are associated with higher levels of ACE2 in the brain, which might contribute to the higher risk of CNS SARS-CoV-2 infection in cognitively impaired individuals and AD patients.

Keyword: *ACE2, Alzheimer's disease, Blood-brain barrier*

#54 Immunomodulatory therapies interfere with meningeal inflammation and subependymal injury in CNS autoimmunity.

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[2]McGill University, Montreal, Canada,
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People living with multiple sclerosis (MS) experience episodic central nervous system (CNS) white matter lesions instigated by autoreactive T cells. With age, MS patients show evidence of grey matter demyelination and experience devastating non-remitting symptomology. What drives progression is unclear and has been hampered by the lack of suitable animal models. We have shown that passive experimental autoimmune encephalomyelitis (EAE) induced by an adoptive transfer (A/T) of young Th17 cells induces a non-remitting clinical phenotype that is associated with persistent leptomeningeal inflammation and cortical pathology in old, but not young SJL/J mice. Here we show that the SJL/J A/T EAE model in aged mice also replicates thalamic atrophy with an ependymal-in gradient of microgliosis and astrogliosis. In addition, we show that immunomodulatory therapies not only

reduce the number and size of leptomeningeal immune cell aggregates but also improve cortical and thalamic pathology, including demyelination, microgliosis and astrogliosis.

Keyword: *EAE, meningeal inflammation, thalamus, immunomodulatory therapy*

#58 Characterization of the transcriptional response of human brain derived endothelial cells to pro-inflammatory cytokines IFN-gamma and TNF-alpha in vitro

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CRCHUM, Montreal, Canada

Background: Multiple sclerosis (MS) is an auto-immune disease of the central nervous system (CNS), characterized by a disruption of the blood-brain barrier (BBB) and the meningeal brain barrier (BMB). The mechanisms leading to barrier degradation in MS involve effects of secreted cytokines/chemokines on the endothelial cells (ECs), thereby leading to CNS immune infiltration. Exposure of the endothelium to pro-inflammatory cytokines interrupts the homeostasis of the barriers by disrupting tight and adherent junctions, consequently increasing the permeability of the barrier. Cytokines secreted by different effectors of the immune system induce various functional responses in ECs, quantifiable through measurement of the transcriptome.

Objectives:
1: Compare the transcriptomes of BBB and BMB-associated ECs in steady-state.
2: Identify the transcriptional response of ECs to cytokine stimulation.

Methods: ECs from human brain tissue removed at surgery were put in culture and inflamed with cytokines (TNFα and IFNγ). Bulk RNA-Seq was performed on treated and untreated EC with paired-end fragments on an Illumina NextSeq500 machine, at a targeted depth of 50M reads per sample. Reads were aligned to the human reference genome with STAR. Differential expression analysis was performed with R package limma. Biological insights from sets of differentially expressed genes were obtained through gene-set enrichment analysis.

Results: Preliminary analysis on three samples per group showed significant transcriptomic differences between the ECs forming the BBB and the BMB. We found that BMB and BBB differed in their immunological profiles for the expression of cell adhesion molecules, chemokines and cytokines such as IL11 and CCL26. Moreover, cytokine stimulation induced significant transcriptional changes in the BBB/BMB-associated ECs. These changes included genes such as SOD2, IL32 and IRF1. Overall, ECs' response to cytokine stimulation involved both immune pathways (e.g. defense response, inflammatory response) and house-keeping functions (e.g. chromosome segregation, tissue development).

Conclusions: Bulk RNA-Seq is a powerful sequencing approach for homogenous samples such as ECs in culture. This allowed to identify transcriptional signatures associated with the anatomical source of these cells (BBB vs BMB), demonstrating that there are differences in the molecular properties of ECs depending on the structure they are forming. Gene expression changes induced by cytokine stimulation showed that ECs are highly responsive to cytokine stimulation, suggesting they are central to the processes that lead to immune infiltration and have an important role in mediating neuroinflammatory events.

Keyword: *CNS, Biomarkers, Bioinformatics, blood-brain barrier, endothelial cells*

#63 Mannose Receptor C Type 2 is a Promoter of Inflammatory and Invasive CD8+ Cytotoxic T cells in Multiple Sclerosis

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[3]*Université de Montréal / Faculty of Medicine and Centre de Recherche du CHUM, Neuroscience, Montréal, Canada,*
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Background

Preventing pathogenic immune cell migration across the blood brain barrier (BBB) is amongst the most efficient therapeutic approach to treat patients with acute multiple sclerosis (MS). Leukocytes need to degrade extracellular matrix (ECM) molecules at the basal lamina of the BBB to reach the deep brain parenchyma, a hall mark in MS pathology. Intriguingly, mannose receptor C type (MRC)2 is responsible for the degradation of collagen IV, the most abundant ECM molecule at the BBB. Therefore, we hypothesize that MRC2 is involved in trans-endothelial migration of pathogenic leukocytes across the BBB and might be an attractive therapeutic target to treat MS.

Objectives

1) To analyze whether MRC2 expression is altered on circulating peripheral blood mononuclear cells (PBMCs) and brain-infiltrating leukocytes in MS. 2) To determine whether MRC2 expression is altered upon in vitro polarization on CD4+T(h)elper and CD8+T(c)ytotoxic cell subpopulations. 3) To assess the contribution of MRC2 in BBB-trafficking of pathogenic leukocytes in the context of MS.

Methods

Confocal microscopy on post-mortem brain tissue derived from MS patients. Flow cytometry analysis of PBMCs from healthy controls (HC) and MS, and in vitro polarized Th1, Th2, Th17 and Tc1, Tc2, Tc17 cells. In vitro migration assays using primary human brain endothelial cells and PBMCs from healthy donors and MS patients treated with specific MRC2 blocking antibody or isotype control, followed by flow cytometry analysis.

Results

MRC2 is significantly co-localized on perivascular accumulating CD8+Tc cells in active MS lesions on post-mortem brain tissue from 5 MS patients. MRC2 is specifically upregulated on in vitro polarized pro-inflammatory CD8+ and CD4+ subpopulations (Th/c1), but not on anti-inflammatory Th/c2 cells. CD14+ monocytes show highest MRC2 frequency among immune cells, whereas MRC2 frequency and expression (geoMFI) are significantly elevated on MS-derived CD8+ cells in comparison to HC. Comparison of gene expression profiles of MRC2+CD8+Tc cells and MRC2-CD8+Tc cells highlights that MRC2 is significantly linked to an invasive and pro-inflammatory phenotype. Our in vitro migration assays indicate that specific blocking of MRC2 interferes with CD14+ monocytes and pro-inflammatory CD8+ T cell subpopulations (TNF+CD8+ and IL17A+CD8+).

Conclusions

MRC2 appears to play a role in trans-endothelial migration of CD14+ monocytes and particularly of pro-inflammatory, invasive CD8+Tc cells in the context of MS. Further confirmation of migration assays and ongoing in vivo studies using the experimental autoimmune encephalomyelitis mouse model will elucidate whether MRC2 indeed represents a novel therapeutic target to

interfere with MS disease development and progression.

Keyword: *Blood-brain barrier, Extracellular matrix degradation, CD8+ T cells*

#75 Impacts of pro- and anti-inflammatory cytokines on the blood-brain barrier and the blood-meningeal barrier

Irem Akbulak[1], Stephanie Zandee[1], Antoine Fournier[1], Pénélope Dessay[1], Skander Ben Ahmed[1], Carla Rodriguez Mogeda[1], Fiona Tea[1], Camille Grasmuck[1], Karine Thai[1], Wendy Klement[1], Lyne Bourbonnière[1], Sandra Larouche[1], Alain Bouthillier[3], Robert Moumdjian[3] and Alexandre Prat[1,2]

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Background: Many cytokines are found within multiple sclerosis (MS) lesions. Endothelial cells (EC) in brain and meninges are affected by cytokines secreted by glial and infiltrating dendritic cells, macrophages, monocytes and T cells. Few of these cytokines were studied at the blood-brain barrier (BBB) and blood-meningeal barrier (BMB) levels. This project aims to study the effects of pro- and anti-inflammatory cytokines on BBB and BMB in normal conditions and MS.

Objectives & Aims: Explore alterations in immune cell migration, examine the effects of pro- (IL-17, IFNg, TNFa, GM-CSF, IL-6, IL-23, IL-1b) and anti-inflammatory (IL-10, TGFb, IL-35, IL-4) cytokines on EC, study the production of cytokines and chemokines by EC stimulated by these cytokines, characterize the endothelial cytokine receptors, identify alterations in tight junctions (TJ) and adherence junctions (AJ) to unravel the role of cytokines in MS development and progression.

Methods: Bulk RNA-sequencing and qPCR were used to identify TJ and AJ expression (PECAM, beta and delta catenin, ZO1, occludin, claudin-5, JAM-1) in cytokine-stimulated BBB and BMB-EC. Protein levels of the TJ/AJ were confirmed using Western Blot and immunofluorescence. Cellular adhesion molecules (CAM) MCAM, VCAM, ICAM, ALCAM, avb3 were studied using flow cytometry and confocal microscopy. Trans-endothelial electrical resistance assay was used to study the effect of cytokines on the integrity of the BBB/BMB-EC. Additionally, a previously published sc-seq dataset from our lab, encompassing mouse EC at the peak of experimental autoimmune encephalomyelitis, was queried for TJ/AJ/CAM/receptor expression.

Results: Not all pro- and anti-inflammatory cytokines influence the BBB and BMB in the same way. Additionally, the BBB and BMB can react differently to the same cytokine. Furthermore, some cytokines mainly affect TJ/AJ, whereas others affect CAMs more. Surprisingly, TGFb mostly down-regulates TJ/AJ on both BBB and BMB, whereas IL-4 increases these molecules. IL-1b and IFNg/TNFa had the strongest upregulating effect on ICAM and VCAM expression, followed by IL-17.

Conclusions: Cytokines can have a versatile effect on the BBB and BMB, and they very likely tightly regulate the access of circulating immune cells to the CNS. Understanding the impact of cytokines implicated in MS lesions on the BBB/BMB can help us better understand the processes of immune cell migration and potentially help us identify new targets for therapy.

Keyword: *Neuroinflammation, Blood-brain barrier, Cytokine*

#88 In vivo imaging how the brain barriers establish CNS immune privilege

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University of Bern, Theodor Kocher Institute

The meninges at the outer border of the central nervous system (CNS) contribute to maintaining CNS protection and immunity. How the leptomeningeal layers control access of immune cells and immune mediators into the CNS is however not well understood. Here we show junctional localization of VE-cadherin in arachnoid and pia mater fibroblasts bordering the cerebrospinal fluid (CSF) filled subarachnoid space (SAS) and that VE-cadherin$^+$ arachnoid and pial fibroblasts form adherens junctions. In vivo imaging of the brain and spinal cord in VE-cadherin-GFP knock-in reporter mice allowed for direct observation of accessibility of CSF derived soluble mediators or immune cells to CNS compartments beyond the SAS during health and neuroinflammation as well as of detection of volume changes of the SAS during autoimmune CNS pathology. VE-cadherin-GFP reporter mice furthermore allowed to identify Prox-1+/VE-cadherin-GFP+ cells as a cellular layer of the arachnoid mater. Taken together, we have identified VE-cadherin as a novel landmark for in vivo imaging of the leptomeninges as a prerequisite to visualizing the borders of the SAS and thus potential barrier properties of the leptomeninges in controlling access of immune mediators and immune cells into the CNS during health and neuroinflammation.

Keyword: *leptomeninges, experimental autoimmune encephalomyelitis, cerebrospinal fluid, subarachnoid space, two-photon imaging*

#109 The Role of Ano1, a Voltage-Sensitive Calcium-Activated Chloride Channel, on Blood-Brain Barrier Endothelial Cells and Astrocytes during Neuroinflammation

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The loss of blood-brain barrier (BBB) integrity is a hallmark of multiple sclerosis (MS). Identifying novel key players involved in this process is thus crucial for the development of MS therapies. The goal of this research project is to provide evidence that Anoctamin-1 (Ano1) is involved in early astrocyte and BBB-endothelial cell (BBB-EC) activation, contributing to the weakening of the BBB.

Immunohistofluorescence staining of mouse and rat central nervous system (CNS) tissue has demonstrated the presence of Ano1 on endothelial cells and astrocytes. A comparative analysis further demonstrated that Ano1 is upregulated on both cell types following active experimental autoimmune encephalomyelitis (EAE) induction in WT B6 mice and Sprague Dawley rat. These observations were confirmed in vitro by stimulating primary cultures of BBB-ECs and astrocytes with TNF, IFN-gamma and IL-1beta. Pharmacological inhibitors and activators of Ano1 were also tested on cell cultures to assess their effects. Of note, EACT, an activating molecule, induced a downregulation of tight junction and adherens junction molecules, while Ani9, an inhibitor, modulated the downregulation of junctional proteins triggered by inflammatory cytokines. Furthermore, the injection of Ani9 reduced the average clinical score observed and the number of immune cells infiltrating the CNS of WT B6 used in adoptive transfer EAE experiments, as compared to controls. Finally, microscopy imaging and RT-PCR also

demonstrated the presence of Ano1 on human BBB-ECs both in vitro and in situ, while primary cultures of human BBB-ECs were shown to upregulate Ano1 under inflammatory conditions, correlating with the results obtained in rodents.

While additional experiments are needed to further characterize the role of Ano1 in BBB-ECs and astrocytes, these preliminary results provide evidences that modulating Ano1 and its function can have an important impact on neuroinflammation. Furthermore, the similarities in the data collected from human and rodent experiments allow to infer by association that Ano1 inhibitors could potentially also reduce neuroinflammation in humans.

Keyword: *transmembrane protein 16A (TMEM16A), anoctamin-1 (Ano1), blood-brain barrier endothelial cells, astrocytes, Ca2+-activated chloride channels*

#200 Impact of stress-induced inflammation on blood-brain barrier transport

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Major Depressive Disorder (MDD) is a serious chronic mental condition affecting over 280 million people worldwide. This complex and heterogenous illness can be triggered by environmental factors, such as stress. Unfortunately, 30-50% of individuals with MDD do not or respond poorly to available treatments targeting neuronal dysfunction. Intriguingly, resistance to treatment is often associated with an exacerbated immune response as measured by high levels of circulating proinflammatory cytokines such as interleukin (IL)-1, -6 and Tumor Necrosis Factor TNF-alpha (TNFa). Stress-induced inflammation in the brain and subsequent mood alterations may be promoted by the passage of peripheral immune mediators from the periphery into the CNS. Indeed, blood-brain barrier (BBB) alterations are observed following chronic stress exposure in mice, like in the chronic social defeat stress (CSDS) paradigm, a mouse model of depression, as well as in the MDD brain. The BBB is formed by endothelial cells, pericytes and astrocytes and while allowing nutrient exchange from the blood to the brain it also prevents entry of potentially harmful substances. Loss of BBB integrity may contribute to maladaptive stress responses and human depression, but the mechanisms leading to stress-induced BBB alterations and potential as a therapeutic target remain to be determined. We have preliminary data suggesting that caveola-mediated transport of the BBB is altered after CSDS in male and female mice. To gain mechanistic insights and evaluate how stress-induced inflammation modulates BBB dynamic transport and cell signaling, I took advantage of in vitro mouse and human BBB-related cell lines. Endothelial cells were subjected to an immune challenge via treatment with TNFa and expression of genes linked to BBB properties (tight junctions, growth factors, transporters, etc.) were analyzed at various time points. BBB permeability and healing capacity was also evaluated using transendothelial electrical resistance measurement and a scratch assay, respectively. Treatment with other cytokines associated with chronic stress exposure is ongoing along with morphological studies. Overcoming the BBB to treat the brain has been a challenge for decades in psychiatry. Investigating BBB transport mechanisms involved in depression and its modulation in response to stress, inflammation and antidepressant treatments could lead to a better understanding of MDD and novel therapeutic avenues.

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Keyword: *Blood brain barrier, Inflammation, Major depressive disorder, Stress, Transport*

#202 VEGF-E attenuates infarct progression after stroke by promoting stable microvascular remodeling

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Ischemic stroke constitutes a major cause of death and disability of the adult in Canada. Upon stroke, pro-angiogenic responses are activated as an attempt to promote tissue vascularization and subsequent repair. It has been shown that a higher vascular density in the brain correlates with a reduced damage after stroke. Elevated vascular endothelial growth factor (VEGF) levels are associated with attenuated infarct progression due to the formation of collateral vasculature. These observations highlighted the promises of stimulating tissue vascularization through angiogenesis in ischemic stroke therapy. Nonetheless, administration of the potent angiogenic factor VEGF-A increases the risk of vascular destabilization. VEGF-E, a VEGF-A-like isoform encoded by Orf virus, has been shown to stimulate the formation of stable cutaneous vasculature network via activation of divergent signaling pathways in comparison to VEGF-A. Herein, we aimed to evaluate the therapeutic potential of VEGF-E in ischemic stroke. For this purpose, C57BL6/J mice subjected to ischemic stroke via transient middle cerebral artery occlusion (MCAo) were intranasally infused with VEGF-E, 24 and 72 hours after stroke and euthanized 24 hours after last infusion. VEGF-E reduced infarct size and neuronal degeneration as well as extravasation of blood-borne IgG into the injured tissue, translating a reduced vascular permeability. These changes were associated with an increased microvascular density at the lesion site and improved motor recovery. The increased microvasculature density correlated with enhanced covered with pericytes and astrocytes end-feet, which jointly play a key role in vascular stabilization. Attenuation of neuronal damage upon VEGF-E infusion was accompanied with a decreased reactivity of astrocytes in the peri-infarct region outlining a reduced neuroinflammatory response. Our results suggest that VEGF-E infusion into the brain constitutes a promising approach to attenuate ischemic damage by safely promoting injured tissue vascularization while conserving vascular integrity.

Keyword: *Ischemic Stroke, Therapeutic Angiogenesis, Vascular endothelial growth factor*

#219 Identification of sex-specific vascular and immune blood biomarkers of mood disorders

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Major depressive disorder (MDD) and bipolar disorder (BD) will affect approximately 1 in 4 people worldwide. Unfortunately, available treatments focusing on impaired neuronal function are helpful for only 30-50% of individuals with 30% successfully remitting. It suggests that other biological systems may be involved in depression pathophysiology and treatment response. Other plausible causal mechanisms are exacerbated inflammation and neurovascular dysfunction. Indeed, circulating inflammation is elevated in treatment-resistant individuals and neurovascular adaptations modulate cognition, mood, and stress responses. Loss of blood-brain barrier (BBB) integrity has been implicated in affective disorders by our group and others, in both animal models and MDD postmortem brain samples. We propose that identification of biomarkers related to BBB hyperpermeability, and inflammation could help guide mood disorders diagnosis and choice of treatment. Indeed, we observed that blood levels of e-selectin and platelet-derived growth factor (PDGF), two vascular biomarkers providing an indirect measurement of BBB integrity, are altered in mood disorders. Serum samples of 40 women and 62 men from the Signature BioBank (CIUSSS Montreal-Est), one of the largest banks of biological, psychosocial, and clinical data in mental health, were analyzed. It included samples from individuals with a diagnosis of MDD (different levels and comorbidities), BD, or no mental health condition (controls). The Patient Health Questionnaire (PHQ-9, depression state) was performed during sample collection. Blood serum levels of e-Selectin and PDGF were analyzed by ELISA, along with a C-reactive protein (CRP). Comparisons were made by sex and between mental health status. In addition, the PHQ-9 score was used to evaluate the correlational relationship between biomarkers and the severity of depressive symptoms. As expected, controls were characterized by low PHQ-9 scores, individuals with MDD had high scores and BD, with no specific tendency for low or high scores. Both e-selectin and PDGF measurements revealed baseline sex differences, an increase in women with a diagnosis of MDD but not for women with BD. Intriguingly, mood disorders are associated with sex-specific symptomatology, prevalence, and treatment responses. These results indicate basal sex differences in vascular biomarkers, and that blood signatures can be associated with each mood disorder.

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Keyword: *Major depressive disorder, Bipolar disorder, Patient Health Questionnaire 9, Blood-brain barrier*

#227 Adolescent stress increases circulating inflammation altering blood-brain barrier development and maturation

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According to the WHO, major depressive disorder (MDD) is the first cause of disability worldwide with a prevalence at 3-8% in adolescents. Unfortunately, around 40% of depressed adolescents do not or respond poorly to available neuron-centric treatments suggesting that causal mechanisms remain untreated. Chronic stress is an important risk factor to develop MDD and it is associated with increased peripheric inflammation during adolescence. A prolonged rise in inflammatory molecules circulating in the blood can damage the blood-brain barrier (BBB), a highly selective barrier protecting the brain. Intriguingly, adolescents suffering from MDD have elevated plasma levels of markers associated with BBB permeability. Since adolescence is a critical time window for neurovascular development and maturation of the BBB, I investigated how chronic stress exposure impacts it. To do so, I took advantage of an emotional stress paradigm, social instability stress, which induced anxiety- and depression-like behaviors in adolescent male and female mice as measured with behavioral tests. I next explored the link between stress responses and expression of genes associated with BBB integrity and function as well as inflammation (proinflammatory cytokines). Transcriptomic profiling is currently complemented by immunostaining, microscopy and morphological analysis of the neurovascular network and BBB-related cells. Blood serum corticosterone and inflammation level will be assessed by ELISA and a milliplex panel, respectively, to confirm stress-induced activation of the hypothalamic-pituitary axis and immune response and possibly highlight sex differences. Deciphering stress-induced immune and neurovascular alterations occurring during adolescence could allow a better comprehension of the biological mechanisms underlying the development of depression in this understudied population.

#228 Cell type-specific meningeal gene expression signatures in progressive multiple sclerosis

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Meningeal inflammation is a key feature of multiple sclerosis (MS), a prototypic inflammatory disease of the central nervous system (CNS) characterized by compartmentalized lesion pathology and

progressive neurodegeneration. The meninges are an important tissue compartment consisting of both blood-brain and blood-cerebrospinal fluid barriers. Previous studies in progressive MS have highlighted that meningeal immune cell aggregates and subpial cortical pathology are often associated with each other. However, the current understanding of the homeostatic and MS meningeal tissue environment and how a disturbed microenvironment favors chronic inflammation and tissue damage in MS is still limited.

Here, we performed bulk and single-nucleus RNA sequencing of 14 meningeal tissue blocks obtained from 7 people with MS (pwMS) and 7 matched control donors. We utilized various bioinformatic tools including ligand-receptor and differential gene expression analyses and validated results with fluorescence multiplex in situ RNA hybridization and immunohistochemistry.

Specifically, we generated a comprehensive transcriptomic map of the human meninges focusing on stromal, vascular and immune cells. We identified distinct subtypes of each of these cell types including homeostatic and MS-enriched fibroblast subtypes, arterial, capillary and venous endothelial cell subtypes as well as myeloid and dendritic cells, CD4 and CD8 positive T cells, B cells and plasma cells. We verified their spatial expression with RNA and protein-based readouts. Further, with a focus on cell-cell-interaction analysis, we identified specific communication pathways between endothelial and immune cells, highlighting ACKR1 signaling among other pathways associated with a disturbed blood-brain barrier.

Our findings demonstrate specific ligand-receptor interaction pairs between endothelial and immune cells, which might play important roles in immune cell infiltration of the CNS and the promotion of chronic meningeal inflammation and subpial tissue damage. Further research on these interactions is needed to highlight their clinical significance and enable the development of new targeted therapies.

Keyword: *Multiple Sclerosis, Meninges, Neuroinflammation*

#238 Single cell characterization of human meninges in chronic multiple sclerosis.

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Chronic multiple sclerosis (MS) is associated with the accrual of meningeal inflammation and organized ectopic lymphoid tissue. These meningeal infiltrates preferentially affect the arachnoid layer, but whether MS also manifests in the dura layer of the meninges remains controversial. This is despite evidence that the non-diseased dura contains B cell progenitor cells and – likely – also hematopietic stem cells.

Here, we performed a single nuclei RNA-sequencing (snRNA-seq) characterization of meningeal preparations from human donors with chronic MS and controls. We identified novel gene sets defining dura and arachnoid fibroblasts and arachnoid barrier cells and humans. In chronic MS, dura fibroblasts were among the cell types responding most vigorously to the disease. They acquired a pro-inflammatory state with transcriptional signs of hematopoiesis-supporting potential. These observations were confirmed by combining spatial transcriptomics with multi-color RNA-in situ hybridization.

We thus provide evidence that all meningeal layers respond to chronic MS and could locally support lymphopoiesis under inflammatory conditions.

Keyword: *dura, meninges, multiple sclerosis, single nuclei RNA-seq*

#245 Understanding stroma cells of rodent meninges at single cell resolution

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The central nervous system (CNS) is ensheathed by tri-layered meninges. Although the meninges have traditionally been viewed as a purely protective barrier, it is now known that they also play critical roles in immune surveillance, neuronal and glial function, and autoimmune diseases of the CNS such as multiple sclerosis (MS).

Whether the debilitating CNS autoimmune disease MS and its animal model manifest preferentially in the arachnoid or dura layers of the meninges remains controversial. Recent studies provided a detailed single-cell characterization of leukocytes in the rodent dura and demonstrated that B cells and their progenitors reside in the dura, supporting a complex adaptive immune environment. But an extensive analysis of the meningeal non-immune stroma cells is lacking.

Here, we used single nuclei transcriptomics to characterize all cells in murine meninges in health and in an animal model of MS. We identified novel gene sets defining dura fibroblasts, arachnoid fibroblasts, and arachnoid barrier cells, and confirmed the cell-type specific expression of selected markers by RNA-insitu hybridization and immunofluorescence staining. Moreover, experimental autoimmune encephalomyelitis (EAE) - the animal model of MS - induced a pro-inflammatory state in dura fibroblasts and a loss of 'arachnoid-ness' and transcriptional signs of barrier-capacity in arachnoid fibroblasts. Taken together, we constructed a single-cell atlas of all cells in the meninges in health and EAE and found evidence that all meningeal layers react to neuroinflammation during EAE.

Keyword: *single nuclei RNA-seq, meninges, multiple sclerosis, CNS border compartments, stroma cells*

#254 The Skull Periost — Immune Relevant Tissue?

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Introduction:
The brain is enveloped by cerebrospinal fluid (CSF), meninges, and the skull containing bone marrow and attached skull periost. The CSF and meninges host complex tissue-resident leukocytes that protect the brain from blood-borne pathogens and can contribute to brain autoimmunity. Unlike the meninges, tissue immunity in skull periost has not yet been explored.

Aim:
We characterized tissue-resident immunity in rodent skull periost.

Methods:
We first extracted nuclei from freshly isolated skull periost of wildtype mice and performed single nuclei RNA sequencing. Second, we

performed flow cytometry to phenotype extra-vascular leukocytes in the skull periost of wildtype mice after excluding intra-vascular cells with intravenous injection of fluorophore-labeled CD45 antibody and intracardiac perfusion. We deeply characterized the leukocytes in dura, skull periost, and long bone periost of wildtype rats by flow cytometry to achieve greater cell numbers.

Results:
Combining single cell transcriptomics with flow cytometry, we discovered that skull periost in mice contained extra-vascular leukocytes. These were preferentaillly myeloid lineage cells and the myeloid-to-lymphoid ratio was higher in skull periost than in dura. This was confirmed in rats. Marker expression indicated that these leukocytes were preferentially macrophages.

Conclusion:
These findings suggest that skull periost may be an immunologically active tissue and our understanding of CNS associated border compartments could extend to extracranial tissues.

Keyword: *skull periost, single cell RNA-seq, meninges, CNS border compartments*

#262 High-field MRI-guided immunohistochemistry implicates ependymal dysregulation in the emergence of periventricular pathology in the multiple sclerosis brain

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"Surface-in" gradients of pathology are well-documented in the subpial and periventricular regions of multiple sclerosis (MS) brain tissue and suggest a role for cerebrospinal fluid (CSF) in disease pathogenesis. This hypothesis is particularly compelling given MS CSF is known to possess a gamut of immune cells, proinflammatory cytokines, and coagulation factors such as fibrinogen, which could theoretically diffuse into the brain to cause damage. What remains less explored, however, is precisely how these cytotoxic factors gain access to the parenchyma. Damage to the ependyma, which normally forms a semipermeable metabolic barrier at the ventricular border, could provide a logical route for proinflammatory solute entry in MS. Yet, the extent of ependymal cell dysregulation in the MS brain has not been investigated. Using high-field (7T) ex vivo MRI analysis of human MS brain tissue, complemented by MRI-guided immunohistochemistry, we show evidence of ependymagliosis directly adjacent to regions of periventricular demyelination. These reactive ependymal cells are draped in fibrinogen and appear to express fewer junctional proteins. Our data provide strong evidence to suggest that there is ependymal pathology in the MS brain and point to a potential mechanism for the emergence of periventricular "surface-in" gradients.

Keyword: *ependymal cell, multiple sclerosis, periventricular pathology*

#291 Antigen-specific interactions between endothelial cells of the blood-brain barrier and naive CD8 T cells

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Endothelial cells of the blood brain barrier (BBB-EC) are able to present self-antigens but also to cross-present antigens from the periphery or from the brain parenchyma in an MHC class I-dependent manner.
It has been shown that antigen-specific interactions between activated CD8 T cells and BBB-EC are key drivers of the central nervous system inflammation in pathologies including cerebral malaria or Susac syndrome. However little is known about antigen-specific interactions between BBB-EC and naive CD8 T cells, in non-inflammatory conditions.

We used an original mouse model, in which BBB-EC conditionally and inducibly express the influenza virus hemagglutinin (HA) protein as well as a TCR transgenic mouse line in which >95% of CD8 T cells recognize the HA512-520:K^d complex. In in vitro co-culture experiments, we show that BBB-EC expressing HA are able to directly activate HA-specific naive CD8 T cells. Using adoptive transfer of naive CD8 T cells in mice expressing or not HA in BBB-EC, we observed specific induction of activation and proliferation of HA-specific CD8 T cell in HA-expressing recipients. This antigen-specific interaction leads to an accumulation of HA-specific CD8 T cells in the brain parenchyma. These brain-infiltrating CD8 T cells are persisting in the central nervous system up to 30 days after injection and, based on their expression of CD69 and CD103, they seem to acquire a tissue resident memory phenotype in the brain.

The consequences of these interactions and the persistence of a CD8 T cell population in the brain will be assessed, notably on further brain inflammation and on aging and cognitive abilities.

Keyword: *Bood-brain barrier, Antigen presentation, Tissue resident memory T cells, CD8 T cells*

#352 Ependymal interferon-gamma receptor 1 activation plays a crucial role in mediating ependymagliosis and periventricular barrier integrity

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The presence of periventricular lesions (PVLs) in multiple sclerosis (MS) are well-established; however, the underlying cause of this distinct localisation remains less clear [1]. It is thought that cerebrospinal fluid (CSF) might drive these lesions due to the observation that these lesions often appear with a CSF surface-in gradient of damage, and the fact that MS CSF has increased levels of circulating cytotoxic components [1][2][3]. Interestingly, ependymal cells, which are the only cells that separate PVLs from the CSF, display signs of pathology in MS patients, but how ependymal cells are involved with CSF-driven PVL formation is not known [1]. Single-cell RNA-sequencing of MOG-EAE brains demonstrated that ependymal cells upregulated genes associated with interferon-gamma (IFN-gamma) receptor 1 (IFNGR1) activation. This, combined with other unpublished observations in the Stratton Lab, motivated us to investigate the role of IFN-gamma-mediated ependymal alterations

in the context of inflammation. We utilized intracerebroventricular (ICV) injections of IFN-gamma combined with a conditional knockout (cKO) of IFNGR1 in ependymal cells. Our current data suggests a loss of ependyma function in response to IFN-gamma. Specifically, we demonstrate evidence of fluorophore-conjugated dextran infiltration into the periventricular space (administered ICV) at 48h following IFN-gamma, which is reversed in IFNGR1 cKO mice. Our findings suggest that IFN-gamma may be a critical cytokine that may drive ependyma permeability, which could contribute to the formation of CSF-driven PVLs in MS. We aim to substantiate this work by further assessing levels of various other markers of ependyma damage to better understand cellular pathology and the exact pathways underlying functional deficits.

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Keyword: *ependymal cells, IFN-gamma, IFNGR1, MOG-EAE, multiple sclerosis*

#367 Fear conditioning induces sex-specific changes in the blood-brain barrier and vascular system.

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Experiences are linked to emotions impacting memory consolidation and associated brain neuronal circuits. Posttraumatic stress disorder is an example of strong negative emotions affecting memory processes by flashbacks of past traumas. Stress-related memory deficits are also observed in major depressive disorder (MDD). We recently highlighted that stress induces blood-brain barrier (BBB) alterations in a sex and brain region specific manner in mice and human depression. However, little is known about the relationship between emotional valence, memory encoding and BBB function.

In this study, we evaluated the effects of negative emotional valence through an aversive memory experience: fear conditioning. We evaluated the impact of this paradigm on BBB properties in brain regions related to memory and emotions processes. Male and female mice went through the fear conditioning paradigm, which includes trials (sound and footshocks) on day 1, context, cue and recall tests in the following 3 days. We observed sex differences in multiple behavioral and biological variables: females showed more freezing as well as higher circulating corticosterone levels as compared to male mice. Comparison of ventral hippocampus and

prefrontal cortex BBB transcriptomes revealed striking increased genes expressions and variations between sexes. Fibroblast growth factor 2, which is known to regulate BBB tight junctions' expression, was upregulated in some brain regions, such as ventral hippocampus, amygdala and prefrontal cortex, for both male and female mice in a drastic way for mice receiving footshock. We also measured central and peripheral levels of pro- and anti-inflammatory cytokines, including interleukin (IL)-6, IL-1b and Tumour Necrosis Factor (TNF)-a, to investigate immune responses and how could it be correlated to memory experience, BBB genes expression and corticosterone levels in the blood.

In summary, mice that experienced acute stressful situation showed several BBB changes, that are different between male and female animals, which gives us additional information on the role of the BBB integrity in memory formation following traumatic events.

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Keyword: *Blood-brain barrier, fear conditioning, cytokines, hippocampus, corticosterone*

CNS-infiltrating innate immune cells

#48 Innate lymphoid cells of the CNS: Functional and phenotypical characterization along ontogeny.

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Infiltrated and resident innate lymphoid cells (ILCs) are present in both, parenchymal and non-parenchymal structures of the murine central nervous system (CNS). ILCs are very plastic cells capable of integrating into the tissue and rapidly respond to environmental cues. Nevertheless, the dynamics of the CNS-ILC niche establishment and the functionality of these CNS-derived ILCs remain unclear.

We showed that the brain of adult young mice contained quiescent tissue resident group 1 ILCs (NK cells and ILC1s) and ILC2s but almost no RORgt-expressing ILC3/LTis. In contrast, in RORgt-deficient adult animals, anomalies affecting the dura lymphatic vessels were observed. These alterations determined by confocal microscopy and RT-qPCR were not observed in animals lacking T cells (Rag1-knockout), indicating a possible involvement of RORgt-expressing ILC3/LTis in dura lymphatic formation along CNS development.
The investigation of the ILC niche formation during ontogeny indicated that their infiltration was initiated during embryonic development (E14-E16), when Id2$^+$ILCs were observed to start seeding the brain. ILC infiltration occurred before T cells arrival into the CNS, and both, PLFZ$^+$PD-1$^+$ ILC progenitors and lineage-committed ILCs were already detected at E16. ILC numbers increased as the brain develops, displaying subset-specific dynamics of tissue-seeding. Furthermore, the brain ILC niche development seemed to depend on in-situ proliferation during prenatal stages, and on peripheral cell infiltration postnatally.

In contrast to the adult brain, in the prenatal CNS not only group 1 ILCs but also RORgt$^+$ILC3s and LTi cells were detected, while ILC2s arrived after birth. Importantly, functional ILC3s and LTi were located within newborn brains and dura meninges but disappeared during the first weeks of life. Perinatal ILC3s and LTi expressed molecules involved in synaptic transmission (Nrgn, Ddc) and lymphoid tissue formation (Tnfsf11, LTa, LTb), which could explain the lymphatic alterations observed in RORgt-knockout animals. Additionally, an in situ phenotypical plasticity (ILC3->ILC1) was observed already at perinatal timepoints as the RORgt$^+$ cells progressively disappear.

In conclusion, tissue resident ILCs infiltrate the brain early during development. Resident ILCs display functional phenotypes that suggest their implication in CNS homeostasis and development of the dura lymphatics.

Keyword: *Innate lymphoid cells (ILCs), Tissue-residency, ILC3/LTi cells, Dura lymphatic vessel, Ontogeny*

#151 Infiltrating myeloid cells expressing the protein Ninjurin-1 exacerbate RR-EAE.

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Multiple Sclerosis (MS) is an autoimmune disease characterized by the demyelination of neurons in the central nervous system (CNS) and subsequent neuroinflammation. A main cause of this neuroinflammation is peripheral immune cells entering the CNS via the blood-brain barrier (BBB). One way these immune cells get access to the brain is through adhesion molecules expressed on the BBB. Nerve Injury-Induced Protein- 1 (Ninjurin-1) is a homophilic adhesion molecule that is highly expressed on myeloid cells and BBB endothelial cells (ECs). Previous data showed that Ninjurin-1 plays a role in the rodent model of MS, Experimental Autoimmune Encephalomyelitis (EAE), in the chronic form of disease. Using the relapse-remitting model of EAE (RR-EAE), we analyzed Ninjurin-1 expression on myeloid cells at different phases of the disease: pre-onset, onset, peak, and remission. In addition, we characterized the phenotype of

Ninjurin-1$^+$ cells in the spleen and CNS of EAE mice. Moreover, to understand the factors driving Ninjurin-1 expression we incubated BBB-ECs and myeloid cells with both anti- and pro-inflammatory cytokines. Finally, in order to determine the role played by Ninjurin-1$^+$ cells during disease, we injected EAE mice with an anti-ninjurin-1 peptide at peak and at remission. The expression of Ninjurin-1 on myeloid cells significantly increased in the CNS during onset and peak of RR-EAE. These Ninjurin-1$^+$ infiltrating myeloid cells expressed significantly more co-stimulatory molecules and cytokines when compared to Ninjurin-1$^-$ cells. The levels of Ninjurin-1 expression on BBB-ECs and CD11b$^+$ myeloid cells were both increased in CD4-TH1 inducing conditions. Ninjurin-1 blockade at both peak and remission of disease significantly reduced the clinical score of the treated mice when compared to the control. Our data demonstrates Ninjurin-1 plays a critical role in neuroinflammation and confirms the therapeutic potential of anti-Ninjurin-1 in EAE.

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Keyword: *Ninjurin-1, EAE, Peripheral Myeloid Cells, Inflammation*

#169 SUSTAINED INFILTRATION OF NEUTROPHILS INTO THE CNS RESULTS IN INCREASED DEMYELINATION IN A VIRAL-INDUCED MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS) characterized by neuroinflammation and immune-mediated axonal demyelination. Current disease modifying therapies target T-cells and B-cells to decrease relapses and disease severity, but these alone do not arrest disease progression and highlight a need for identifying other cellular mediators of pathology. The present study examines the molecular contributions by which neutrophils augment demyelination in a viral-induced model of MS. Intracranial inoculation of the neuroadapted JHM strain of mouse hepatitis virus (JHMV) into susceptible strains of mice results in acute encephalomyelitis and chronic immune-mediated demyelination similar to that of MS.

JHMV infection of transgenic mice (Tg mice), in which expression of the neutrophil chemoattractant chemokine CXCL1 is under the control of a tetracycline-inducible promoter active within GFAP-positive cells, results in sustained neutrophil infiltration of the central nervous system (CNS) that correlates with increased spinal cord demyelination. Immediately prior to onset of demyelination in this model, flow cytometric analysis of CD45+ cells in Tg mice compared to controls revealed differences in neutrophil, macrophage, and CD8+ T cell infiltration, suggesting a role for neutrophils in amplification of inflammation and recruitment of demyelination-associated cell types. Additionally, flow cytometric and single cell RNA sequencing (scRNAseq) analysis at peak demyelination demonstrated increases in activated neutrophils as determined by altered neutrophil morphology, protein expression, and increased expression of mRNA transcripts associated with increased neutrophil effector functions, such as CD63, MMP9, S100a8, S100a9, and ASPRV1. Collectively, these findings reveal insights into the role of neutrophils in recruitment of cell types associated with demyelination onset and into changes in the profile of neutrophils associated with increased white matter damage in mice persistently infected with a neurotropic coronavirus.

#188 Bone marrow-derived dickkopf-1 promotes brain injury progression after ischemic stroke

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Introduction: Ischemic stroke is a leading cause of death and disability among adults in Canada. The circulating levels of dickkopf-1 (DKK1), an endogenous secreted protein that acts as a potent inhibitor of the canonical Wnt pathway, are elevated in stroke patients correlating with a poor prognosis. Little is known about the mechanisms underlying the contribution of DKK1 to stroke pathobiology. Dkk1 gene is not enriched in the brain, but its expression is detected at the lesion site after experimental stroke, outlining a peripheral source. Indeed, immune cells in the bloodstream have been proposed as a major source of DKK1. **Hypothesis and objectives:** Our study postulates that DKK1 is produced by bone marrow-derived cells (BMDCs) that infiltrate the brain, hindering tissue regeneration by deregulating the canonical Wnt pathway. **Methodology:** To test this hypothesis, chimeric mice in which the expression of DKK1 is induced specifically in BMDCs were subjected to ischemic stroke using middle cerebral artery occlusion (MCAo). **Results:** Our findings indicate that DKK1 is essentially released by BMDCs that are recruited to the lesion site 1 week after ischemic stroke. We reveal the recruited DKK1-inducible BMDCs exhibit myeloid origin to the lesion site. Interestingly, DKK1 induction in BMDCs reduces vascular β-catenin mRNA expression, outlining canonical Wnt pathway deactivation. Importantly, DKK1 induction in BMDCs deregulates astroglial scar and exacerbates of tissue loss. **Conclusion:** Our findings suggest that BMDCs are a major contributor to DKK1 bioavailability at the lesion site and that its release by these cells play a substantial role in exacerbating injury progression. This knowledge yields a new direction to study DKK1 as a relevant therapeutic target to improve recovery after ischemic stroke.

#278 Identifying molecular mechanisms which activate interleukin-1 beta independently of the inflammasomes in a mouse model of multiple sclerosis

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Multiple sclerosis (MS) is a neurodegenerative disease affecting more than 2 million people worldwide. The autoimmune character of MS is well defined and is the source of many treatments targeting immune cells of people living with the disease. These treatments slow down the disease course, but in return generate an increased vulnerability to infectious diseases without curing MS. It is thus imperative to identify new therapeutic targets to impede MS progression while limiting the risks of harmful and potentially lethal side effects in patients. Using the experimental autoimmune encephalomyelitis (EAE) mouse model, we and others have demonstrated that interleukin-1 beta (IL-1 beta) is a key inflammatory mediator in the pathophysiology of EAE, as mice genetically invalidated for the Il1b gene are protected from disease. To exert its proinflammatory role, the protein requires two distinct signals: a first one which stimulates the transcription of the immature form of IL-1 beta (pro-IL-1 beta), and a second one which activates the canonical pathway of inflammasomes, leading to the cleavage of pro-IL-1 beta into its active form. We hypothesize that blocking all mechanisms of IL-1 beta maturation, whether inflammasome-dependent or not, in all IL-1-beta-producing cells will alter EAE progression and alleviate its symptoms. This hypothesis was tested through three specific objectives. First, we investigated the role of inflammasomes in EAE mice without canonical inflammasome activity. These mice were protected from EAE disease only when depleted in monocytes, suggesting that these cells harbor inflammasome-independent mechanisms activating IL-1 beta. Thus, we next aimed to understand how mouse monocytes cleave IL-1 beta independently of inflammasome activity in vitro and found that monocytes deficient in canonical inflammasome activity can produce active IL-1 beta when incubated in the presence of endothelial cells. And third, to identify the enzymes cleaving IL-1 beta in an inflammasome-independent manner, we created a molecular tool taking advantage of proximity-dependant biotinylation. We confirmed its effectiveness in HEK294 and HeLa cell lines, and now aim to deploy this tool in RAW264.7 macrophage cell line and primary mouse monocytes to unveil the molecular mechanisms leading to IL-1 beta activation in EAE. Together, these results could reveal potential therapeutic targets that could lead to the discovery of an effective and safe treatment for MS patients.

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Keyword: *Multiple Sclerosis, Blood-brain barrier, Macrophage, Functionnal Proteomics*

#292 Monocyte infiltration at early stage of injury induces inflammatory response in an NMO-like mouse model.

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Neuromyelitis optica (NMO) is an autoimmune inflammatory disease that affects the spinal cord and optic nerves of the central nervous system (CNS). It is characterized by the presence of IgG antibodies against astrocyte aquaporin 4 (NMO-IgG). Microglia/macrophages are known to accumulate at the active lesion and are believed to play a role in the disease process. However, the specific functions of microglia/macrophages in NMO have not been extensively studied. Therefore, this study aimed to analyze the functions of monocytes/microglia in an NMO-like mouse model by utilizing homeostatic microglia-specific markers (P2ry12).

An NMO-like mouse model was established by intrastriatal injection of 3 µl of isolated patient NMO-IgG (50 mg/ml) with 5% human complement. The mice were divided into three groups: normal, control-IgG, and NMO-IgG. Various analyses, including histological analysis, gene expression studies, FACS analysis, and rotarod tests, were conducted. The mice were sacrificed at 1 day, 3 days, and 1 week after injection. Monocyte/macrophage depletion was achieved using clodronate, and the effects on pro-inflammatory factors and demyelination were assessed using LFB stain. CD11b+P2ry12-Ly6G-Ly6Chigh monocytes were sorted from the blood and brain of normal and NMO mice, respectively, and subjected to RNA sequencing (RNAseq) analysis.

The NMO groups exhibited a lower latency to fall compared to the other groups in the rotarod test at 1 day. The pro-inflammatory cytokines TNFα and IL-1β were increased at 1 day, and infiltration of CD11b+CD45high monocytes/macrophages was observed. The RNAseq results demonstrated that the infiltrating monocytes immediately differentiated into inflammatory macrophages. Treatment with clodronate reduced the expression of iNOS, IFNr, IL1b, and demyelination, indicating the contribution of monocytes to inflammation and demyelination. In addition, clodronate treatment affect the polarization and function of microglia, which exhibited a high level of inflammatory function regardless of the presence of monocytes.

This study successfully distinguished between monocytes and microglia and analyzed their functions using the microglia-specific marker P2ry12. The findings indicated that infiltrating monocytes play an important role in inflammatory reaction during the early stages of injury.

Keyword: *Neuromyelitis optica, Monocyte, Inflammation, Microglia*

#323 Brain catecholaminergic neurons control monocytes deployment to sites of injury and their loss exacerbate cognitive deterioration in an animal model of Alzheimer's Disease

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The central nervous system (CNS) and the immune system serve as vigilant monitors of the organism's state, orchestrating adaptive responses to maintain homeostasis including the brain itself. In this study, we investigated the potential role that the communication between the brain and the spleen, which is known as the major secondary lymphoid organ in the body.

Intriguingly, we observed impairment in this communication pathway in the 5xFAD Alzheimer's disease (AD) model; disrupting this communication pathway at an early stage of the disease significantly accelerated disease manifestations. We further found that the key players affected by the denervation in this experimental setup are the monocytes, known as pivotal players in coping with neurodegenerative diseases and acute CNS damage. Specifically, we found that as a result of the early denervation, the monocytes exhibited reduced infiltration into the brain of 5xFAD mice, and microglial maturation was adversely affected under these conditions. The impact of the denervation on the ability to cope with degenerative conditions was found to be independent of the primary cause of the conditions. Thus, we found that it was reproduced in a model of retinal injury, in which the protective effect of monocyte infiltration was previously shown. Following spleen denervation, a reduced number of infiltrating monocytes was observed in the injured retinas, further reinforcing the crucial role of the brain-spleen axis in tissue repair processes.

These findings reveal a novel aspect of the intricate relationship between the brain and the immune system, specifically in the context of Alzheimer's Disease. Further research is needed to uncover the precise molecular and cellular mechanisms governing the brain-spleen axis. By elucidating the mechanisms through which the CNS influences immune responses, new therapeutic avenues may be explored to modulate these interactions and potentially ameliorate the impact of AD and other CNS injuries.

Keyword: *Monocytes, Alzheimer's Disease, Brain-Immune Interactions*

#328 Implication of Dual Immunoglobulin Domain containing Cell Adhesion Molecule in Myeloid Cell Migration to the Central Nervous System in Multiple Sclerosis

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Background

Multiple sclerosis (MS) disease is characterized by the blood-brain barrier (BBB) and brain-

meningeal barrier (BMB) disruption and lesion formation due to leukocyte infiltration into the central nervous system (CNS). The leukocyte infiltration is mediated by the cell adhesion molecules (CAMs), which promote the extravasation of peripheral blood mononuclear cells across the CNS microvasculature. Among the CAMs, Dual immunoglobulin domain containing cell adhesion molecule (DICAM) is expressed on pathogenic immune cell subsets such as $CD4^+$ T cells. Furthermore, in our lab we have also determined DICAM expression on myeloid cells (CD14+ monocytes and CD11c+ dendritic cells) that play an important role in T cell activation and thus contribute to brain inflammation. Building on these results, we are now investigating the role of DICAM in myeloid cell migration during inflammation.

Objectives

To investigate DICAM expression on myeloid cells and its role in their migration across the BBB and BMB in vitro.

Methods

To study DICAM expression on peripheral blood mononuclear cell derived monocytes, and cultured macrophages and dendritic cells from MS patients as well as healthy subjects, we performed flow cytometry using specific panels. We also used immunohistofluorescence (IHF) to localize DICAM+ myeloid cells in the brain and identify the type of lesions where DICAM is expressed. IHF also allowed us to compare DICAM expression in the MS patient brain with patients of amyotrophic lateral sclerosis that we consider as other neurological disease control. To unravel DICAM's role in human monocyte migration, we performed in vitro Boyden migration assays as well as Flow adhesion assays mimicking the blood flow.

Results

Flow cytometry on cultured DCs and macrophages allowed us to quantify DICAM expression and compare DICAM expression on these cell subsets between MS patients and healthy donors. The IHF results unveiled the type of lesions where DICAM+ myeloid cells are localized the most during inflammation and in steady state.

We found a higher frequency of $DICAM^+$ monocytes in relapsing remitting, secondary progressive and primary progressive MS patients when compared to healthy subjects. Strikingly, the frequency of DICAM monocytes was significantly higher in MS patients with active disease. Furthermore, SPMS patients have a higher frequency of DICAM dendritic cells compared to healthy donors. In vitro flow adhesion assays showed that blocking DICAM reduced monocyte rolling and adhesion on BBB ECs by 33%, whereas migration assays revealed a 22% decrease in monocyte migration on BMB ECs and 19% on BBB ECs after DICAM blockade.

Conclusion

Our results suggests that DICAM plays an important role in myeloid cell migration into the CNS and possibly affects disease progression. DICAM blockade can reduce myeloid cell migration across the brain barriers and could therefore decrease lesion formation.

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Keyword: *DICAM, Blood brain barrier, Inflammation, Migration, Myeloid cells*

#341 Central nervous system fibroblast activation induced by neurotropic coronavirus infection

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The central nervous system (CNS) contains meningeal and perivascular fibroblasts which are quiescent during homeostasis but activate during inflammation. Activated fibroblasts form an extracellular network and produce chemokines and adhesion molecules for immune cell recruitment. If chronically activated, such as during multiple sclerosis (MS) or neuroborreliosis, these fibroblasts can drive ectopic lymphoid follicle formation. These ectopic follicles are positively correlated with disease in MS and represent a potential therapeutic target. The goal of this work is to define the cells and signals responsible for meningeal fibroblast activation using the murine hepatitis virus (MHV) model of CNS infection. MHV infection activates meningeal fibroblasts which produce chemokines required to recruit T cells and control infection. Intriguingly, IgD^+IgM^+ B cells emerge early in the CNS during MHV infection and other models of neuroinflammation; however, their role is unknown. B cells signal to stromal cells in lymphoid organs through lymphotoxin alpha1beta2 (LT); therefore, we hypothesized that early recruited IgD^+IgM^+ B cells participate in meningeal fibroblast activation through LT. Histological staining of meningeal fibroblasts indeed revealed upregulation of podoplanin (PDPN), a marker of fibroblast activation at 3 days post infection in proximity to IgD^+ B cells. LT agonism enhanced MHV induced chemokine upregulation, however, B cell depletion did not alter LT expression, stromal cell-derived chemokine mRNA levels, or T cell recruitment. As macrophages can also activate fibroblasts via TNF, anti-CSF1R antibody was administered to ablate macrophages, the predominate immune cell recruited early after infection. Anti-CSF1R treated mice revealed impaired LTβ mRNA upregulation in the CNS, albeit no effects on Pdpn or chemokine mRNA levels. Our results support that myeloid cells, but not B cells, participate in partial meningeal fibroblast activation and that fibroblast network activation may be a cooperative multi-step process involving multiple players.

#342 Targeted Depletion of Arginase-1 Expressing Myeloid Cells Exacerbates Neuroinflammation in Experimental Autoimmune Encephalomyelitis

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Background: A significant proportion of the inflammatory cells that infiltrate the central nervous system (CNS) of patients with multiple sclerosis (MS) and animals with experimental autoimmune encephalomyelitis (EAE), are

myeloid cells. A large body of literature supports a pathogenic role of these subsets during neuroinflammation. However, we and others have shown that CNS-infiltrating myeloid cells are more heterogeneous than traditionally thought, and that their phenotype evolves throughout disease. Arginase 1 (Arg1) is a canonical marker of alternatively activated myeloid cells (AAMC) with anti-inflammatory properties. Arg1+ myeloid cells accumulate in the CNS during EAE, but are not detectable in the blood or peripheral tissues. In MS, myeloid cells expressing AAMC markers predominate the quiescent core, as opposed to the active demyelinating rim, of lesions.

Objective: The objective of this study is to determine the role of AAMCs in the resolution of inflammation and recovery of neurological function in a relapsing-remitting model of EAE.

Methods: We constructed a transgenic mouse strain with the human diphtheria toxin receptor (DTR) gene (Hbegf) linked to the Arg1 promoter (Arg1DTR mice). In order to restrict DTR expression to hematopoietic cells we reconstituted lethally irradiated WT mice with bone marrow cells from $Arg1^{DTR}$ donors. EAE was induced in $Arg1^{DTR}$ →WT chimeric mice by the adoptive transfer of myelin-reactive CD4+ Th17 cells and recipients were treated with diphtheria toxin (DT) or vehicle. CNS inflammatory cell composition was evaluated via flow cytometry and cell subsets were isolated via FACS for transcriptional analysis. Pathological features were assessed by immunohistochemistry.

Results: Depletion of Arg1+ inflammatory cells in $Arg1^{DTR}$ chimeric mice exacerbated EAE and resulted in a high mortality rate compared with PBS-treatment and WT→WT chimeric controls. Monocyte-derived dendritic cells possessing an anti-inflammatory phenotype were most susceptible to depletion. The more severe clinical course observed following depletion was associated with an increase in Arg1- inflammatory macrophages in the spinal cord, coincident with elevated levels of proinflammatory cytokines and chemokines in spinal cord homogenates. Conversely, genetic ablation of Arg1 in immune cells had no impact on EAE incidence or severity.

Conclusions: This study suggests that CNS accumulation of AAMC during later stages of autoimmune demyelinating disease suppresses the local inflammatory response and ameliorates neurological deficits by an Arg1- independent mechanism.

Keyword: *myeloid cells, EAE, Arginase-1, Neuroinflammation*

#370 ILC1 trafficking upon Toxoplasma gondii infection

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Innate lymphoid cells (ILCs) are highly abundant at mucosal surfaces contributing to immediate immune responses in response to invading pathogens. In the steady state, comparably low numbers of ILCs are found in the central nervous system (CNS). However, the abundance of e.g. ILC1s and NK cells strongly increases in the course of cerebral T. gondii infection. To clarify whether these cells are derived from local precursors or are recruited from the periphery, mice with cerebral toxoplasmosis were treated with S1PR agonist FTY20 to block ILC recruitment to the site of inflammation. Here we show that FTY720 treatment reduced the frequency of circulating

ILCs in the blood. In the brain, numbers of T cells were significantly reduced while number of ILC1s and NK cells increased. Despite lower numbers of T cells in the brain, host defence against parasite was not impaired.

Keyword: *cNK, ILC1, FTY720, T. gondii*

Emerging therapies for neuroinflammatory conditions

#14 REcTOs proteins: new tools for the treatment of chronic inflammation

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Background - Dysregulated cytokines boost the inflammatory reaction and enhance tissue damage in multiple sclerosis. The mRNA turnover of several cytokines and chemokines is post-transcriptionally controlled by RNA binding proteins (RBPs). RBPs act as physiological brakes targeting proinflammatory cytokines messengers and inducing their degradation. Tristetraprolin (TTP) is a RBP that targets transcripts encoding several proinflammatory cytokines, including TNFa, IL1-b, IL-6 and GM-CSF. Regnase1 is another RBP that binds and degrades targets that include IL-6, IL-12p40 and IL-17 [1, 2]. RBPs are negatively regulated by the same inflammatory pathways that control immune cells activation, therefore, like other physiological brakes, RBPs undergo inactivation during chronic inflammation.

Materials & Methods - We designed and generated hybrid proteins (REcTOs) that harbor the RNA binding domain of TTP along with the RNase domain of Regnase1. By immunofluorescence we compared the cellular distribution of REcTO proteins to the parental proteins. We evaluated the binding of REcTOs to TNFa mRNA using RNA immunoprecipitation and by ELISA assays we studied the effect of REcTOs on TNFa levels on LPS-treated RAW264.7 cells. Among four REcTO proteins we selected REcTO4 and generated lentivirus encoding REcTO4 allowing the study of this chimera in in BV2 cells, U-937 cells and Bone Marrow Derived Macrophages (BMDMs). RNA-seq transcriptome analysis was also performed on RAW264.7 cells transduced with REcTO4.

Results - REcTO4 displayed a cytosolic distribution that overlapped both TTP and Regnase1. REcTO4 efficiently binds and degrades the mRNA of TNFa. In several myeloid cell lines treated with LPS, REcTO4 significantly reduces the levels of secreted TNFa, IL-6, GM-CSF and IL-1b. The transcriptome analysis of REcTO4-expressing RAW264.7 cells showed that REcTO4 significantly down-regulates gene expression of inflammatory pathways induced by LPS, as well as TTP target transcripts such as Tnfa, Il6, Il-1b,Csf, Il10, and Zfp36 [3]. Finally REcTO4 can downregulate TNFa in BMDMs in which we did in vitro knockout of TTP.

Conclusions - REcTO proteins are a novel and efficient tool to regulate the production of inflammatory cytokines. Being composed of TTP and Regnase1 domains that do not contain regulatory elements that inhibit both TTP and Regnase1 expression and functionality. Thus, cell transplantation coupled to REcTO gene therapy could represent a new therapeutic approach to target neuroinflammation

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Keyword: *TTP, RNA binding proteins, neuroinflammation, gene therapy*

#26 Intravenous delivery of AAV encoding a scFv antibody targeting TDP-43 mitigates symptom phenotypes and pathology in a mouse model of vascular dementia.

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Vascular dementia is the second most common type of dementia after Alzheimer's disease. This progressive disease is due to pathological reduction in cerebral blood flow. Some studies reported that Chronic Cerebral Hypoperfusion (CCH), one of the major causes of vascular dementia, is associated with neuronal damages, especially in the cortex and hippocampus regions and which leads to cognitive disorders. But the mechanistic link between the cerebrovascular pathology and the cognitive impairments remains elusive and currently, no cure or effective treatment for vascular dementia does exist.

Recently, our lab generated a CCH mouse model by UCCAO (Unilateral Common Carotid Artery Occlusion)[1]. The mice subjected to UCCAO surgery exhibit severe TDP-43 pathology in cortical neurons, with cytoplasmic mislocalization of TDP-43, formation of insoluble phospho-TDP-43 aggregates, neuroinflammation and the development of cognitive and motor deficits.

Considering the pathological changes TDP-43 distribution and the cognitive impairment observed in the CCH mouse, we have tested the therapeutical potential of an adeno-associated virus (AAV) vector encoding a scFv (single-chain variable Fragment), called VH7Vk9, composed of the heavy and light chain hypervariable regions of a monoclonal antibody that binds specifically TDP-43.

Here, we used a new version of the AAV vector, bearing a recombinant capsid (B10) designed to achieve efficient neuronal transduction after injection directly into the bloodstream and allows efficient blood-to-brain transfer. The AAV-scFv anti-TDP-43 (and an AAV-scFv anti-GFP used as a control) were bilaterally injected intravenously in the retro-orbital sinus of CCH mice. This resulted in significant pan-neuronal expression of the encoded scFv in brain and spinal cord. We report that the intravenous injection of this AAV-delivered scFv anti-TDP-43 in CCH mouse model improved cognitive impairments and motor deficits caused by CCH. Moreover, expression of the scFv VH7Vk9 Ab was able to reduce the number of cortical neurons with cytoplasmic TDP-43 aggregates.

Thus, intravenous administration of a single dose of AAV-B10 encoding scFv anti-TDP-43 Ab led to sustained production scFv Abs in neurons with ensuing beneficial effects in a mouse model of vascular dementia. The results support the view of a key role for TDP-43 in pathogenic changes associated with brain hypoperfusion and they

suggest that antibody approaches targeting TDP-43 might provide new therapeutics for vascular dementia.

Keyword: *ALS, intrabodies, scFv, TDP-43, in vivo models*

#35 Administration of anti-TDP-43 antibody improves symptom phenotypes and reduces TDP-43 pathology in mouse model of sporadic ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of motoneurons. Abnormal cytoplasmic aggregates of TDP-43 (TAR DNA-binding protein 43) are a pathological hallmark of ALS, in both familial and sporadic cases. Recently, our group (Pozzi et al.,2019; 2020) reported that an antibody (called E6 Ab) directed against the RRM1 (RNA Recognition Motif 1) domain of TDP-43 mitigated TDP-43 pathology in transgenic mice expressing TDP-43 mutants linked to familial ALS. Here, we tested the effects of E6 Ab in a new mouse model of sporadic ALS based on intracerebroventricular (i.c.v.) infusion of CSF from sporadic ALS patients in mice expressing the human wild type TDP-43 protein (hTDP-43WT) (Mishra et al., 2020). Intrathecal injection of E6 Ab ameliorated motor performance and reduced TDP-43 proteinopathy in the lumbar spinal cord. Remarkably, the co-infusion of E6 Ab i.c.v. with ALS-CSF using mini-osmotic pump for two weeks in 8-month-old mice expressing hTDP-43WT mitigated TDP-43 pathology and rescued motor and cognitive deficits in this mouse model of sALS. These results suggest that injection in the CSF of antibodies targeting TDP-43 is an approach that should be considered for treatment of ALS and other neurodegenerative disorders with TDP-43 pathology.

Keyword: *Amyotrophic Lateral Sclerosis, TDP-43, antibodies, human CSF*

#39 Interferon-alpha receptor antisense oligonucleotides reverse neuropathology in a mouse model of type I interferon neurotoxicity

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Chronic production of the antiviral cytokine interferon-alpha (IFN-alpha) in the brain is neurotoxic. This is best observed in patients with genetic cerebral interferonopathies such as Aicardi-Goutières syndrome, where chronic intrathecal production of IFN-alpha causes debilitating disease and premature death. There is no cure for these diseases with existing treatments largely aimed at ameliorating symptoms. Thus, a novel therapeutic strategy is urgently needed. Here, we investigated the potential of antisense oligonucleotides targeting the murine IFN-alpha receptor (mIfnar1 ASOs) in a mouse model for cerebral interferonopathies. Intracerebroventricular injection of mIfnar1 ASOs into transgenic mice with brain-targeted chronic IFN-alpha production resulted in a blunted cerebral interferon signature, reduced neuroinflammation, restoration of blood-brain barrier integrity, absence of tissue destruction and lessened neuronal damage. Remarkably, mIfnar1 ASO treatment was also effective when given after onset of neuropathological changes, reversing some of the features, showing a reversible aspect of IFN-alpha-mediated neuroinflammation and neurotoxicity. We

conclude that ASOs targeting the IFN-a receptor halt and reverse progression of IFN-a-mediated neuroinflammation and neurotoxicity, opening a new and promising approach for the treatment of patients with cerebral interferonopathies.

Keyword: *interferonopathy, Antisense-oligonucleotides, therapy, interferon-alpha, Aicardi-Goutieres syndrome*

#52 Harnessing a beneficial regulatory immune response with salbutamol

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Multiple sclerosis (MS) is an immune-mediated disease that attacks the central nervous system (CNS) leading to abnormalities in movement and sensory functions. Lesions in the CNS show immune cell infiltration, demyelination, and axonal damage. Over time, repair of lesions in the CNS may occur but is often inadequate. This could be a contributing factor to the worsening of disability in people living with MS. We aim to identify new avenues for promoting recovery in the CNS. Previous work (Mishra et al., J Neurosci 2021) found that microglia/macrophages stimulated with LPS/IL4/IL13 display a marked pro-remyelinating potential in the lysolecithin demyelinating model. cAMP-response element binding protein 1 (CREB1) was identified as an important nexus in programming the regenerative phenotype. To overcome difficulties in using the LPS/IL4/IL13 cocktail as a therapeutic, such as the potential harmful effects of LPS, we seek alternative activators of CREB1 as potential candidates to elicit the regenerative effect of LPS/IL4/IL13. One approach is activating CREB1 through the beta2 adrenergic receptor. Activation of beta2 adrenergic receptor has previously been proposed to have therapeutic potential in MS. Salbutamol is a common bronchodilator used in asthma and COPD and is a selective agonist for beta2-adrenergic receptor. My results show that while salbutamol does not activate macrophages by itself, it strongly altered the inflammatory response to lipopolysaccharide (LPS) stimulation. Addition of salbutamol decreased LPS-induced pro-inflammatory TNFa cytokine level while increasing regulatory features: elevation of IL10 and arginase activity. These changes are also observed in microglia. Salbutamol treatment from induction of experimental autoimmune encephalomyelitis prevented disease development, while treatment from onset of clinical signs have not affected clinical severity likely because the subsequent disability course was too pronounced. These results may form the preclinical bases to translate salbutamol into a clinical trial in MS to ameliorate pro-inflammatory activities while harnessing regulatory molecules to facilitate regenerative processes.

Keyword: *Immunomodulation, Multiple Sclerosis, Neuroinflammation, Recovery, Salbutamol*

#74 Tolebrutinib better exerts an anti-inflammatory effect on human and murine myeloid cells in comparison to evobrutinib, both inhibitors show promise in inducing phagocytic cell repair mechanisms

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Introduction:

Bruton tyrosine Kinase (BTK) is a kinase expressed in immune and neural cells and acts downstream of pattern recognition receptors to activate the NF-kB cascade. In MS, BTK inhibitors (BTKis) are currently in clinical trials as novel disease-modifying therapies. While most research on BTKis is focused on B cells, understanding the impact of BTK inhibition in non-B cell populations

is critical, given many BTKis can exert their effects in both the peripheral and central components.

Objectives/Aims:

Here, we determine how BTK inhibition in human and murine myeloid cells can alter their phenotype and function in the context of MS-relevant inflammation and repair.

Methods:

Human whole blood, monocyte-derived macrophages (MDMs), primary mouse bone marrow-derived macrophages (BMDMs), microglia and mixed glia were pre-treated with evobrutinib or tolebrutinib (0.1nM-10uM), prior to LPS or IL-1B stimulation. TNF, IL-6, IL-10 release was measured via ELISA. MDMs and BMDMs were treated with tolebrutinib or evobrutinib (0.1uM-10uM), prior to LPS stimulation and phosphorylation of BTK was measured via Cell Signalling p-btk ELISA and immunocytochemistry. The phagocytosis rate was measured using pHrodoTM Zymosan beads and quantified with the Cytation Imager.

Results:

Both TNF and IL-6 were decreased in human whole blood pre-treated with BTKis; In human MDMs, TNF and IL-10 were decreased – IL-6 did not. In mouse microglia, TNF was decreased while IL-6 and IL-10 did not. In BMDMs TNF and IL-6 decreased while IL-10 did not. In mouse mixed glia, TNF is decreased using Tolebrutinib stimulated with LPS. Tolebrutinib and evobrutinib are inhibiting the phosphorylation of BTK under inflammatory conditions in MDMs and BMDMs. BTKis increase the phagocytosis rate in MDMs under inflammatory conditions.

Conclusion:

Human myeloid cells are responsive to BTKis and decrease pro-inflammatory cytokine release stimulated by LPS. A species difference is observed with lesser effects achieved in mouse myeloid cells at lower dosing levels. There are baseline levels of p-btk in MDMs and BMDMs and the inhibitors can decrease both inflammatory and baseline phosphorylation levels. Phagocytosis of pHrodoTM beads increases with both Tolebrutinib and Evobrutinib, providing a potential repair mechanism within the central nervous system.

#78 Neuregulin-1 enhances remyelination in progressive demyelinating lesion of cuprizone mice by fostering a reparative phenotype in microglia.

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Funding: MS Canada and the Hillary Kaufman Lerner Memorial Fund

Introduction: Prolonged demyelination in progressive multiple sclerosis (PMS) leads to white matter degeneration and disability accumulation. Currently minimal regenerative treatments are available for PMS. Uncovering the mechanisms of remyelination is crucial to develop effective treatments for PMS. Pro-regenerative microglia facilitate remyelination by clearance and recycling of lipid-rich myelin debris in acute demyelinating lesions. Microglia are abundant in chronic lesions, providing an opportunity to therapeutically harness their potential for remyelination in PMS. We previously discovered that Neuregulin-1 (Nrg-1), a key myelination factor, is declined in MS lesions. Restoring Nrg-1 levels promoted remyelination in acute demyelinating lesions and supported a pro-generative phenotype in microglia. Here, we aimed to determine whether availability of Nrg-1 can foster oligodendrocyte (OL) maturation and remyelination by enhancing microglia capacity

for myelin phagocytosis and cholesterol biosynthesis in chronic demyelinating lesions.

Methods: We induced chronic demyelination by cuprizone (CPZ) diet for 10 weeks in PDGFR-Cre mice to allow tracking of new OLs. PLX5622 [colony stimulating factor 1 receptor inhibitor] was used to deplete microglia in CPZ mice. Nrg-1 treatment was administered by subcutaneous injection. Immunohistochemistry, electron microscopy, behavioral tests, and lipid assessment were performed in CPZ mice. Parallel in vitro experiments were conducted in primary cultures of OL progenitor cells (OPCs) and microglia to assess myelin phagocytosis, lipid metabolism and remyelination.

Results: In vitro, Nrg-1 promotes myelin phagocytosis in activated microglia and elevates the cellular levels of esterified and free cholesterol resulting in an increase in efflux of cholesterol from microglia through upregulation of cholesterol efflux transporter ABCA1. Exposure of OPCs to conditioned media from activated microglia treated with Nrg-1 promoted maturation of OPCs into myelinating OLs. In CPZ mice, Nrg-1 treatment led to a significant increase in remyelination in chronic CPZ demyelinating lesions through microglia-dependent mechanisms as Nrg-1 effects were diminished in PLX5622 treated mice.

Conclusion: We report that Nrg-1 treatment can promote remyelination in chronic demyelinating lesions by enhancing the capacity of microglia for cholesterol metabolism and release. These new findings identify Nrg-1 as a potential treatment strategy for PMS.

Keyword: *Progressive multiple sclerosis, Remyelination, Neuregulin-1, Chronic Cuprizone mouse model, pro-generative phenotype in microglia*

#164 Silybin modulates extracellular, but not intracellular, neurotrophic factors improving motor and inflammatory outcomes in an MPTP-induced Parkinson's disease mouse model.

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Parkinson's Disease (PD) is the most common neurodegenerative movement disorder, affecting over 10 million people worldwide. PD presents cardinal motor symptoms such as tremors, rigidity, postural instability, and other motor and non-motor symptoms. While the precise cause remains unclear, distinct biochemical alterations have been identified, including the degeneration of dopaminergic neurons and reduced dopamine levels within the substantia nigra and striatum, accompanied by pro-inflammatory and oxidative stress.

In the context of PD, disruptions in the activity and levels of neurotrophic factors (NTFs) have been observed and correlated with motor dysfunction severity. These NTFs include insulin-like growth factor-1 (IGF-1), brain-derived neurotrophic factor (BDNF), and glial cell-derived neurotrophic factor (GDNF), which act extracellularly, as well as the mesencephalic astrocyte-derived neurotrophic factor (MANF) and cerebral dopamine neurotrophic factor (CDNF), which act intracellularly. Research suggests that extracellular NTFs play a role in

modulating neuronal death and neuroinflammation, while intracellular NTFs protect from stress responses. Consequently, there is growing interest in treatments that modulate NTF to alleviate PD symptoms and combat neurodegeneration.

Silybin (Sb), the primary bioactive compound in S. marianum, has neuroprotective effects in the MPTP-induced mouse PD model. Sb preserves dopamine content, modulates oxidative stress, reduces neuroinflammation, and restores BDNF content in PD mice. However, the effects of Sb on other NTFs in PD have not been studied. To elucidate the role of Sb, Sb was orally administered simultaneously with the MPTP-induced parkinsonism scheme in mice. Motor behavior was assessed using pole, traction, and beam tests, and a cytokine levels profile was measured for quantifying neuroinflammation. Our results showed that Sb treatment restored BDNF, GDNF, and IGF-1 levels but did not affect MANF and CDNF levels in MPTP-intoxicated mice. NTFs restoration correlated with improved motor behavior in all the motor tests. Additionally, Sb treatment was associated with increased anti-inflammatory responses derived from IL-10, IL-4, and fractalkine. These findings suggest that Sb has the potential to act as a neuroprotective agent by modulating NTFs and improving motor behavior and neuroinflammation outcomes in PD.

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Keyword: *Parkinson's Disease, Silybin, Neurotrophic Factors, Motor Improvement, Anti-inflammatory*

#189 Modulation of motor behavior and immune response by transplantation of induced neural stem cells in multiple sclerosis animal models

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Multiple Sclerosis (MS) is an autoimmune-related inflammatory CNS demyelinating disorder that affects multiple white matter tracts. The goal of current MS treatment is primarily to prevent recurrence without regenerating the affected nerve cells. Our study explores novel cell-based therapies designed to protect neurons from inflammation and replace neurons lost in MS.

We developed an induced neural stem cells (iNSCs) from human fibroblasts using the Sendai virus delivery system containing four Yamanaka factors in our neural induction medium. The iNSCs were induced in approximately 14 days and further expanded 2 weeks more for cell transplantation into the Experimental Autoimmune Encephalomyelitis (EAE) model mice. The iNSCs demonstrated immunopositivity against NSC markers and expressed NSC-associated genes such as Sox1, Sox2, Nestin and MSH1. These cells proliferated beyond 10 passages, maintaining a normal karyotype. After transplantation of the iNSCs, the therapeutic effects were observed in EAE model animals. In the experimental groups transplanted with the iNSCs, the EAE mice showed a superior ability of motor function recovery compared to control group, with recovery degree assessed through an established EAE scoring protocol. Immunostaining assays of brain and spinal cord slices from the EAE model mice revealed that iNSC transplantation enhanced myelination and suppressed inflammation in both the brain and the spinal cord, as compared to control mice receiving saline injections.

Taken together, these results show that transplantation of the iNSCs dramatically improved the motor functions and exerted an anti-inflammation effect in the MS model animals, which suggests that the iNSCs may be feasible as a promising therapeutic cell source for MS treatment.

Keyword: *Multiple Sclerosis, Induced neural stem cell, Cell transplantation, Motor function, Immune response*

#239 Targeting Innate-mediated Signaling for Treatment of Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative disease that affects a significant proportion of the population. Approximately 15% of MS patients suffer from progressive stages of MS, characterized by a gradual and continuous accumulation of

irreversible disability, for which there is currently no effective treatment. While the earlier stages of MS are primarily driven by adaptive immunity, the mechanisms underlying the progressive stages involve innate immune dysfunctions. Precise triggers of these disease stages remain unclear, but emerging evidence suggests that Pattern Recognition Receptors (PRRs), which coordinate the innate immune response, may play a significant role. In our studies utilizing a mouse model of MS, Experimental Autoimmune Encephalomyelitis (EAE), we demonstrated that disrupting certain innate signaling pathways had unexpected impact on disease outcome, with certain molecules driving the disease or providing protection against it. Specifically, our research revealed that mice deficient in specific innate molecules exhibited resistance to EAE, as evidenced by significantly lower clinical scores and restored myeloid cell percentages compared to controls. Interestingly, these molecules have also been shown to be upregulated in peripheral blood mononuclear cells (PBMCs) of MS patients and associated with MS brain lesions. Building upon these findings, we identified an efficient compound that targets a mechanism controlled by innate signaling. We have conducted in vitro studies using mouse and human cells, as well as in vivo experiments. Remarkably, the compound demonstrated nearly 90% efficiency in reducing intended target in human monocytes and conferred protection against severe disease development in mice. Currently, our focus is on understanding the cellular and molecular mechanisms behind the critical role of innate signaling in EAE and MS pathogenesis, as well as testing different compounds. Through additional in vivo studies and therapy administration parameters we hope to provide insights into a promising avenue for developing novel therapeutic options for MS.

Keyword: *Multiple Sclerosis, Pattern Recognition Receptors (PRRs), EAE, Innate Signaling*

#244 Increased signal transduction mediated by extracellular vesicles

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Extracellular vesicles (EVs) have garnered significant attention in the study of oncological and neurological diseases. However, their clinical application has been hindered by challenges in tailoring their cargo and tissue specificity. Nevertheless, their potential as delivery vectors for various molecules remains intriguing, particularly in the context of pathological conditions. In this study, we loaded EVs with IL4 (IL4 EVs) and employed them to induce a phenotypic shift toward an activated state in microglia. To achieve this, we engineered a murine microglia cell line through lentiviral overexpression of IL4 or CRISPR Cas9-mediated tagging of IL4R with eGFP. Notably, IL4 EVs exhibited faster and more potent effects on recipient microglia compared to an equivalent concentration of recombinant IL4 (rIL4) administered in soluble form. The internalization of EVs into recipient cells occurred through endocytosis, enabling the release of cargo into the cell cytosol via endosomal escape. To investigate this process further, we employed electron microscopy and immunofluorescence to examine EV internalization, measuring the colocalization of specific organelles within the endocytic pathway. Our findings suggested that the disparity in signaling efficiency between the

treatments may be attributed to their differential interaction with IL4R in terms of cellular localization and receptor engagement. Additionally, we performed live imaging to study receptor cluster formation following short-term administration of IL4 EVs and rIL4. The results indicated distinct effects of IL4 EVs and rIL4 on recipient cells. In our model, IL4 EVs induced a shift in microglia phenotype within 3 hours of administration, exhibiting faster kinetics compared to rIL4. This disparity suggests differences in the underlying process or components involved in IL4 EVs versus rIL4-mediated effects. Notably, IL4 EVs stabilized receptor cluster formation on both plasma and endosomal membranes while maintaining their numbers over time, contrasting with rIL4 treatment, which increased receptor quantity. Although the clinical use of EVs remains distant, understanding their signaling mechanisms and biology is crucial for advancing research on their potential as delivery tools. Leveraging the advantages observed in vitro, the translation of EV-based therapeutics in vivo could offer novel and highly effective strategies for delivering therapeutic molecules.

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Keyword: *Extracellular vesicles, IL4, Neuroinflammation, drug delivery, receptor clustering*

#252 Vidofludimus Calcium, an Orally Available DHODH Inhibitor in Phase 3 Clinical Trials for Multiple Sclerosis, Potently Activates NURR1

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Background: The orally available dihydroorotate dehydrogenase (DHODH) inhibitory small molecule, vidofludimus calcium (VidoCa), being developed for the treatment of multiple sclerosis (MS) is currently tested in phase 2 and 3 clinical trials for progressive (P)MS and relapsing (R)MS, respectively. Nuclear receptor related 1 (NURR1) is a nuclear receptor (NR) that is highly expressed in neurons and thought to have a neuroprotective function. Also, anti-inflammatory roles of NURR1 in peripheral and brain resident immune cells have been described. Altered NURR1 expression levels have been detected in Parkinson's and MS patients and

heterozygous NURR1 knockout in experimental autoimmune encephalomyelitis (EAE) mice caused earlier disease onset. Here, we investigated the potential of VidoCa to act as NURR1 agonist.

Methods: Nuclear receptor modulation by VidoCa was determined in luciferase Gal4 reporter gene assays containing the NURR1 ligand binding domain (LBD), full-length NURR1 (monomer [NBRE] or NURR1/RXR heterodimer [DR5]) or other NRs. VidoCa binding to NURR1-LBD was evaluated with isothermal titration calorimetry (ITC). The biological effect of VidoCa was assessed in vitro in a glioblastoma cell line, T98G, and in lipopolysaccharide (LPS) stimulated PBMC with/without VidoCa. Expression of the NURR1-regulated genes (tyrosine hydroxylase [TH], vesicular amino acid transporter 2 [VMAT2]) by PCR or brain derived-neurotrophic factor [BDNF] secretion by ELISA were used as readout, respectively. The overall activity of VidoCa on disease activity was assessed in a therapeutic rat EAE model.

Results: NURR1 reporter assays show a ~3-6-fold activation of NURR1 by VidoCa. EC_{50}'s were 0.4±0.2, 0.3±0.1 and 0.4±0.2 microM for NURR1-LBD, -NBRE and -DR5, respectively. ITC analysis showed an affinity (Kd) of 0.7 microM for VidoCa binding to the NURR1-LBD. For the other NRs, VidoCa showed only activity for NUR77 and NOR1 with a ~7-fold lower potency as compared to NURR1. VidoCa showed a dose-dependent upregulation of both TH and VMAT2 in T98G cells. Preliminary results from LPS stimulated PBMC showed a tendency towards increased BDNF levels upon VidoCa treatment. In addition, VidoCa significantly reduced disease severity in the EAE model.

Conclusion: Besides the anti-inflammatory capacity of VidoCa via DHODH inhibition, the additional NURR1 agonism may provide further neuroprotective benefits in patients with MS, which is currently tested in clinical studies for RMS and PMS.

Keyword: *Vidofludimus Calcium, NURR1, DHODH, Multiple Sclerosis*

#265 Tolerogenic dendritic cells manifest strong cellular respiration as evidenced by oxygen consumption and lactate production

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The therapeutic use of antigen-specific tolerogenic dendritic cells (tolDCs) is a promising approach for the treatment of autoimmune diseases such as multiple sclerosis (MS). Despite the rapid increase of clinical studies in the past decade, little is known about their mode of action and optimal culture conditions. As metabolic pathways have been assigned a crucial role in shaping immune responses, the milieu in which tolDCs are generated is likely to affect their tolerogenic state [1, 2]. While the effects of the metabolic microenvironment on conventional dendritic cells (convDCs) are well studied [3], similar studies on tolDCs are limited. We hypothesize that the immunoregulatory effect of tolDCs can be shaped by adjusting the microenvironment during the culture process. In doing so, we aim to enhance monocyte conversion into tolDCs and optimize the manufacturing process for clinical application.

Human convDCs and tolDCs are generated from monocytes in the presence of IL-4 and GM-CSF and stimulated by adding the pro-inflammatory cytokines IL-1-beta, TNF-alpha, and PGE2. The

tolerogenic phenotype of tolDCs is induced by 1,25(OH)2 vitamin D3 and confirmed by allo-MLR and flow cytometry. Next, we monitor metabolically relevant parameters, including oxygen (O2) consumption and lactate production, and assess the effect of different culture conditions such as O2 deprivation and daily media change on the cells' characteristics.

Our data show that tolDCs generated in the presence of 1,25(OH)2 vitamin D3 demonstrate reduced expression of the maturation markers CD80, CD83, and CD86 as compared to convDCs, and induce T-cell hyporesponsiveness in an allo-MLR [4]. Additionally, tolDCs manifest significantly increased lactate production and O2 consumption as compared with convDCs. The highest demand for O2 is established during the differentiation phase and sustained during the subsequent maturation phase. Furthermore, hypoxic culture conditions significantly decrease the viability and conversion rate of the cells, elicit reduced expression of the identity marker CD209 and the maturation markers HLA-DR, CD83, and CD86, and upregulate the migration markers CCR5 and CCR7. Daily removal of acidic metabolites affects the semi-mature phenotype of tolDCs, but not their tolerogenic function.

Our observations may provide further insights into how tolDCs contribute to tolerance induction by metabolomic reprogramming. Since hypoxia has a pronounced effect on DC phenotype, we are currently investigating how this impacts the function of the cells. Furthermore, these findings might offer a deeper understanding of tolDC fate after patient administration, where O2 levels do not exceed 5%. In summary, a better understanding of tolDC metabolism could lead to new ways of optimizing tolDC-based vaccines by increasing their potential to fight autoimmune diseases.

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Keyword: *tolerogenic dendritic cells, tolerance, multiple sclerosis, autoimmunity, cell-based vaccination*

#270 Longitudinal Monitoring of Plasma GFAP and TCRß-Clonotypes Following ATA188 Adoptive T-Cell Therapy

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Introduction: In a Phase 1 open-label trial, treatment with ATA188, an off-the-shelf, allogeneic EBV-specific T-cell immunotherapy, was associated with confirmed disability improvement (CDI) in some patients with progressive MS, supported by less brain atrophy

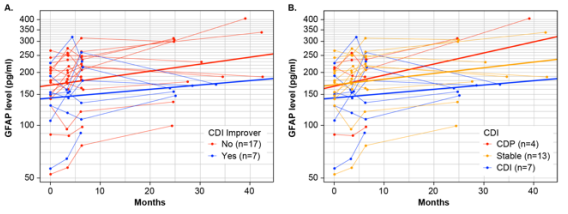
over time. Longitudinal monitoring of glial fibrillary acidic protein (GFAP), an emerging biomarker of progressing neurodegeneration expressed primarily in astrocytes, as well as ATA188-derived EBV-specific TCRβ-clonotypes may provide further mechanistic insights underlying ATA188-associated CDI.

Design/Methods: Plasma GFAP was measured longitudinally using SiMoA technology and then correlated with treatment time in subjects achieving CDI versus not; CDI versus stable versus confirmed disability progression (CDP). Deep sequencing of TCRβ-clonotypes was utilized as a tool to detect ATA188-derived EBV T cells following treatment.

Results: GFAP levels significantly increased during treatment in those not achieving CDI (n=17) but not the CDI group (n=7). Patients with CDP (n=4) showed significantly higher GFAP increases over time versus patients with CDI; there was no significant difference when comparing stable (n=13) versus CDI or stable versus CDP, although the latter showed a trend (Figure 1).
Nineteen of 24 patients showed evidence of ATA188-derived EBV-specific TCRβ-clonotypes, with some exhibiting EBV-encoded LMP2A antigen (HLA A2-restricted, FLYALALLL)-specific reactivity. In some patients, ATA188-clonotypes were detected 2 months after receiving their final dose in year-1, which was the latest time point tested (Figure 2).

Conclusions: Data presented here show that CDI following ATA188 treatment appeared coincident with stabilization of GFAP, while patients with CDP showed significant increase in GFAP over time. Furthermore, ATA188-derived EBV-specific TCRβ-clonotypes were detected up to 2 months post-infusion.

Figure 1. Longitudinal linear mixed model analysis of plasma GFAP levels. *Levels of plasma GFAP significantly increased over time (panel A) in the no CDI group (n=17) but not the CDI group (n=7). Similarly, when the no CDI group was separated into those with stable EDSS (n=13) or those with CDP (n=4), both groups showed a significant increase in GFAP levels over time (panel B) while the CDI group did not. Increases in plasma GFAP over time in the no CDI vs CDI groups were not significantly different (panel A table). However, when CDI and CDP groups were compared, plasma GFAP increases over time were significantly higher in the CDP group compared to the CDI group (panel B table).*



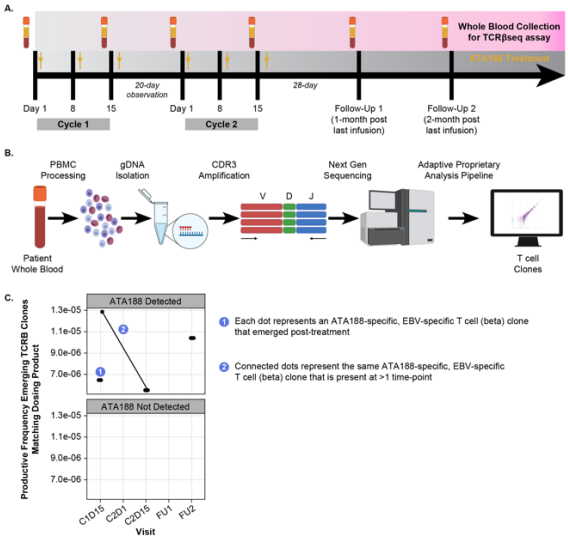
Linear mixed model in:	Slope	P-value	Intercept
CDI - Yes	0.00829	0.395	7.16 (142.9 pg/ml)
CDI - No	0.0134	**0.00204**	7.40 (169.1 pg/ml)

Comparsion	P-value
CDI - Yes vs CDI - No	0.323

Linear mixed model in:	Slope	P-value	Intercept
CDI	0.00829	0.395	7.16 (142.9 pg/ml)
Stable	0.0108	**0.0158**	7.41 (169.9 pg/ml)
CDP	0.0210	**0.0474**	7.38 (167.0 pg/ml)

Comparsion	P-value
CDI vs CDP	**0.0456**
Stable vs CDP	0.0764
CDI vs Stable	0.547

Figure 2. TCRβ-sequencing as a tool to identify ATA188-derived EBV T cells post-infusion. *TCRβ-sequencing of the CDR3 region from patient peripheral blood mononuclear cells collected before, during, and following ATA188 dosing (panel A) were compared against the TCR repertoire profile of the ATA188 infused product they received as well as a public database of putative EBV-specific TCRβ-sequences (panel B). EBV-specific TCRβ-clones fully matching the ATA188 lot and detected only following but not prior to ATA188-treatment, were considered ATA188-clonotypes. Panel C shows representative data depicting two subjects – one subject where TCRs matching ATA188 infused product were detected post-infusion (Panel C, top row) and one where TCRs matching ATA188 were not detected (Panel C, bottom row).*



Keyword: *Cell therapy, Biomarkers, Multiple Sclerosis*

#277 FGFR inhibitor infigratinib prevents hippocampal inflammation and demyelination in a model of multiple sclerosis

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Background. Multiple sclerosis (MS) is the most common demyelinating disease caused by autoimmune, inflammatory mechanisms in the central nervous system (CNS). According to MRI and neuropathology studies, hippocampal lesions are observed early in the disease course correlating with cognitive impairments, which are significantly evident after 5 years of disease progression suggesting a therapeutic window. Current disease-modifying treatments address the inflammatory features of MS through immunomodulation/-suppression but do not target demyelination nor failing remyelination. Upregulation of fibroblast growth factors (FGF) and receptors (FGFR) has been shown to play a key role in establishing and maintaining a demyelinating microenvironment in white matter lesions of MS and its disease model setting the stage for a pharmacological inhibition of FGFR signalling. In experimental autoimmune encephalomyelitis (EAE), FGFR inhibition with the small molecule infigratinib prevented and suppressed severe clinical episodes and was associated with reduced immune cell infiltration, less myelin sheath and axon destruction, enhanced oligodendrocyte maturation and increased remyelination in spinal cord tissue. In this study, we aimed to investigate whether the protective effects of infigratinib on the spinal cord could be reproduced in the hippocampus, as another severely affected CNS region in MS patients.

Experimental approach. The oral FGFR inhibitor infigratinib (30 mg/kg/d) was applied for 10 days after induction of EAE. Hippocampal tissue samples were collected at the peak (day 17 p.i.) and chronic phase (day 41 p.i.) of EAE. Expression of FGFR downstream signalling, remyelination inhibitors and myelin proteins were measured by Western blot. Inflammatory infiltrates, demyelination/myelination, P25 (+) mature oligodendrocytes and NeuN (+) neurons in the cornu ammonis and gyrus dentatus of hippocampal tissue were quantified by histological and immunohistochemical staining.

Key findings. Preventive FGFR inhibition with infigratinib exerted sustained beneficial effects on the hippocampus of EAE-mice. In the acute phase, administration of infigratinib resulted in downregulated FGFR1 (p=0,0047) and FGFR2 expression (p=0,0053) and reduced expression of the remyelination inhibitor SEMA3A (p=0,0017). In addition, reduced inflammatory infiltrates with fewer CD3(+) T cells (p=0,0478), B220 (+) B cells (p=0,0409) and Mac3 (+) microglia (p=0,0206) could be observed in the hippocampus of infigratinib-treated mice. Further, P25(+) oligodendrocyte maturation was increased (p=0,0005) and myelin sheath destruction reduced (p=0,0019) in the white matter of the hippocampus. In the chronic phase of EAE, the FGFR Inhibition led to an upregulation of myelin basic protein (MBP) expression (p=0,0184) accompanied with reduced CD3(+) T cells (p=0,0193), increased P25(+) oligodendrocytes (p=0,0072) and less demyelinating white matter lesions (p=0,203) in the hippocampus.

Conclusion. Consistent with the findings in spinal cord tissue, targeting FGFR signalling in the hippocampus also has anti-inflammatory and myelin protective effects in a disease model of MS. Therefore, pharmacological inhibition of FGFRs by infigratinib may have the potential to prevent or decelerate the progression of hippocampal damage in MS patients if transferability of these effects from EAE to multiple sclerosis is further investigated.

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#297 Siponimod treatment in chronic neuroinflammation: investigation of its effects on pathogenic Th cell populations

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Secondary progressive multiple sclerosis (SPMS) is a chronic neuroinflammatory disease that develops after a relapsing/remitting stage (RRMS). RRMS is associated with active immune processes and drugs targeting lymphocyte trafficking are effective in controlling disease activity. SPMS pathogenesis is poorly understood, but recently siponimod(BAF312; Mayzent), a drug that modulates lymphocyte trafficking via S1P receptors (S1Pr), has shown efficacy on reducing disability progression in SPMS. It remains unclear how this drug differs from the previously tested lymphocyte trafficking modulators, such as the S1Pr agonist fingolimod (FTY720; Gilenya) that is only used for active/relapsing MS. In this study, we investigated some potential mechanisms that could be behind the therapeutic benefit of siponimod in SPMS.

Previously, we described a novel model of autoimmune neuroinflammation, which can act as a mouse analogue of SPMS (Raveney et al., Nat. Commun. 2015). This model presents with late, chronic EAE, which is linked to a pathogenic cytotoxic-like Th cell subset, $Eomes^+$ Th cells, that infiltrates CNS tissue. The importance of $Eomes^+$ Th cells in disease was confirmed by our further work demonstrating that this Th cell subset is associated with progressive disease in human SPMS (Raveney et al., PNAS 2021). We found that both siponimod and fingolimod treatment reduced CNS Th cell infiltration in developing late, chronic EAE, but only siponimod reduced clinical disease (p=0.0033). Furthermore, siponimod, but

not fingolimod was effective as a rescue treatment in established late, chronic EAE. In the CNS, siponimod treatment was associated with a specific reduction in cytotoxic-like Eomes$^+$ Th cells and CCR5$^+$CXCR6$^+$ Th cells, and led to reduction in peptide-dependent IL-17 and IFN-g production. Interestingly, an investigator-initiated study independent of the sponsor (SPMS, n=43; PPMS, n=9) showed siponimod treatment was also highly effective in human SPMS cases that had high levels of Eomes$^+$ Th cells in peripheral blood compared with those that were Eomes-lo (p=0.0082, ROC AUC= 0.8671).

We have directly investigated Th cells in the target organ revealing key pathogenic markers that are reduced by siponimod but unaffected by fingolimod. Understanding pathogenicity of complex CNS-infiltrating Th cell subsets and which cell are altered by particular drug treatments is a key early step on the road to establishing a personalized medicine approach to treating MS.

Keyword: *Secondary Progressive Multiple Sclerosis, T helper cells, Eomes, Siponimod*

#325 Magnetic Resonance Imaging (MRI) Outcomes from the Long-term Extension Study of Tolebrutinib in Participants with Relapsing Multiple Sclerosis: 2-Year Results

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INTRODUCTION: Tolebrutinib is a brain-penetrant inhibitor of Bruton's tyrosine kinase currently under evaluation for multiple sclerosis treatment. In the double-blind phase (DBP) of the phase 2b trial (NCT03889639), tolebrutinib was well tolerated over 12 weeks with dose-dependent reduction in new gadolinium (Gd)-enhancing T1 and new/enlarging T2 lesions. LTS16004 (NCT03996291) is an ongoing long-term safety (LTS) extension study of tolebrutinib in participants who completed the phase 2b study.

OBJECTIVE: Report MRI outcomes at Week (W) 96 (Year 2) in the LTS extension of the phase 2b tolebrutinib trial in participants with relapsing multiple sclerosis.

METHODS: After the last DBP tolebrutinib dose, followed by a variable treatment gap (0–21 W), participants began the LTS extension Part A, where they continued receiving their DBP dose (5, 15, 30, or 60 mg/day) in a double-blinded manner until the phase 3 dose was selected. In the current open-label extension Part B, all participants receive 60 mg/day. MRI outcomes include numbers of new Gd-enhancing and new/enlarging T2 lesions, T2 lesion volume change from baseline, slowly evolving lesions (SEL), and paramagnetic rim lesions (PRL).

RESULTS: 124 of 125 participants treated in the LTS extension completed Part A and transitioned to Part B; 114 (90.5%) remained on study as of 18 February 2022 (W96 cut-off). At DBP baseline, the mean age (standard deviation [SD]) of enrolled participants was 37.7 (9.6) years (range 19–56); 69% were women. Numbers of new Gd-enhancing lesions remained low in the 60/60-mg arm through W96 and were reduced in other arms at W48 through W96 (W96 mean [SD]: 0.85 [2.5], 0.41 [0.91], 0.90 [2.16], 0.31 [0.66] in 5/60-, 15/60-, 30/60-, 60/60-mg arms, respectively). New/enlarging T2 lesion counts remained low for 60/60 mg. T2 lesion volume change remained low for 60/60 mg (W96 vs baseline, mean [SD]: +0.38 [2.11] cm^3). Median (interquartile range) W96

SEL volume was 247.5 (84–420), 258 (66–906), 570 (133.5–1011), and 244.5 (87–939) mm^3 for 5/60-, 15/60-, 30/60-, and 60/60-mg, respectively. PRL count remained unchanged in 18 participants; 2 participants had 1 PRL at baseline but none at W96, and 3 participants had 1–3 additional PRL at W96 vs baseline (none in the 60/60 mg arm).

CONCLUSIONS: New Gd-enhancing lesion counts remained low for tolebrutinib 60/60 mg and were reduced in the lower dose arms by LTS W48 through W96, when all participants had switched to 60 mg.

STUDY FUNDING: Sanofi.

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Keyword: *Bruton's tyrosine kinase, tolebrutinib, MRI, clinical outcomes*

#326 Safety And Clinical Efficacy Outcomes From The Long-term Extension Study Of Tolebrutinib In Participants With Relapsing Multiple Sclerosis: 2.5-Year Results

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BACKGROUND: Phase 2b trial findings showed brain-penetrant Bruton's tyrosine kinase inhibitor tolebrutinib was well tolerated and elicited dose-dependent reductions in new gadolinium-enhancing T1 and new/enlarging T2 lesions in participants with relapsing multiple sclerosis.

OBJECTIVE: To report tolebrutinib's safety and efficacy at Week 120 (2.5 years) in a Phase 2b trial (NCT03889639) long-term safety (LTS) extension (NCT03996291).

DESIGN/METHODS: In LTS Part A, participants continued their core study tolebrutinib dose (5, 15, 30, or 60 mg/day) double-blind until Phase 3 study dose selection (60 mg/day). In Part B, participants received open-label tolebrutinib 60 mg/day. Safety was assessed via adverse events (AE). Efficacy outcomes included annualized relapse rate (ARR) and change in Expanded Disability Status Scale (EDSS) score from baseline.

RESULTS: In the LTS extension, 107 (85.6%) participants have ongoing treatment as of 7 July 2022. Reasons for treatment discontinuation were perceived lack of efficacy (n=5), progressive disease (n=4), participant's decision (n=3), AEs (n=3), immigration (n=2), and planned pregnancy (n=1). At LTS Week 120, no new safety signals have been observed. The most common treatment-emergent AEs (TEAEs) were Coronavirus disease 2019 (24.8% [31/125]), headache (13.6% [17/125]), nasopharyngitis (12.8% [16/125]), upper respiratory tract infection (11.2% [14/125]), cystitis bacterial, arthralgia and back pain (7.2% each [9/125]), and pharyngitis (6.4% [8/125]). There was no observed tolebrutinib dose effect for TEAEs or serious AEs in Part A and no safety signals

emerged for participants switching to tolebrutinib 60 mg/day in Part B. For participants who received tolebrutinib 60 mg/day for >=8 weeks (n=124), ARR was 0.20 (95% Confidence Interval: 0.14, 0.28) and 73.4% remained relapse-free. Mean EDSS remained stable to Week 120.

CONCLUSIONS: Through LTS Week 120, tolebrutinib 60 mg/day continues to demonstrate a favorable safety profile, and is associated with low ARR and stable disability.

STUDY FUNDING: Sanofi.

ACKNOWLEDGMENTS: Editorial support for this encore abstract was provided by Sheetal Maria and reviewed by Renee Nguyen of Sanofi.

DISCLOSURES: JO: Consulting or speaking fees (Biogen Idec, BMS, EMD Serono, Novartis, Roche, and Sanofi) and research support (Biogen Idec, EMD Serono, and Roche). **SS, TL, NS, and TJT:** Employees of Sanofi (may hold shares and/or stock options in the company). **RJF:** Consulting fees (AB Science, Biogen, Celgene, EMD Serono, Genentech, Greenwich Biosciences, Immunic, Janssen, Novartis, Sanofi, and TG Therapeutics) and research support (Biogen, Novartis, and Sanofi).

Keyword: *Bruton's tyrosine kinase, tolebrutinib, clinical trial outcomes*

#350 Zymosan-stimulated neutrophils suppress T cell proliferation and ameliorate CNS autoimmune disease

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Background: Experimental autoimmune encephalomyelitis (EAE) is a multifocal inflammatory demyelinating disease of the central nervous system (CNS), induced by the adoptive transfer of encephalitogenic CD4+ T cells. It is widely used as an animal model of multiple sclerosis (MS). Axonopathy and neuronal loss are prominent pathological features of both EAE and MS. Disease modifying therapies (DMT) that are currently FDA approved for the treatment of MS, reduce annual relapses significantly. However, none reverse existing CNS damage. The more potent DMTs increase the risk of both common and opportunistic infections. There is an unmet need for more effective immunotherapies that mitigate CNS damage with a favorable safety profile.

Objectives: Recently, our lab identified a new subset of alternatively activated, immature $Ly6G^{low}$ neutrophils that possess neuroprotective and neuroregenerative properties (which we have labeled "N_{aa} cells"). We hypothesized that N_{aa} cells could be harnessed to mitigate chronic disability in mice with EAE.

Methods: We obtained N_{aa} by i.p. injecting zymosan into donor mice then performing an i.p. lavage 3 days post-zymosan injection followed by a $Ly6G^{+}$ magnetic bead sort. For the in vivo experiments, N_{aa} were i.p. injected at days 4 and 6 post-encephalomyeltic CD4+ Th17 T cells transfer.

Results: Unexpectedly, intravenous injection of N_{aa} cells at the time of EAE induction significantly delayed clinical onset. These kinetics are more suggestive of an acute immunosuppressive mechanism of action, as opposed to a neuroprotective or pro-regenerative effect. Subsequent experiments revealed that N_{aa} cells directly inhibit the proliferation of encephalitogenic CD4+ T cells in vitro, in a cell-to-cell contact dependent manner. N_{aa} cells also inhibited the proliferation of purified polyclonal CD4+ T cells during activation with plate-bound aCD3 and soluble aCD28. We found that N_{aa} cells constitutively express PD-L1. We are currently investigating the role of those inhibitory

receptors in N_{aa} cell-mediated immunoregulation.

Conclusion: Collectively, our data suggest that N_{aa} cells have the potential to play multiple beneficial roles in neuroinflammatory disease, by curtailing the expansion of encephalitogenic T cells while promoting neuroprotective and reparative pathways.

Keyword: *Neutrophils, EAE, Immunosuppression, T cells*

#358 Unraveling the effects of gamma-band frequency light flickering strategies on cognition, anxiety, and depression-like behavior, as well as microglia in mice

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Gamma oscillations are rhythmic electric waves ranging from 30-90 Hz, usually under the control of assemblies of fast-spiking neurons. These waves can be entrained by sensory stimulation, such as light flickering, at the same band of frequencies. In homeostatic conditions, neuronal circuits synchronization by gamma oscillations is essential for movement, cognition, and emotional regulation. Recent findings have demonstrated a link between abnormal gamma oscillations and the pathogenesis of several neurodegenerative conditions such as Alzheimer's and Parkinson's disease. As a promising strategy, gamma-band light flickering stimulation (LFS) at 60 Hz can re-synchronize the brain in a resonating gamma band of oscillations, which has been shown to improve cognition in patients affected by dementia and age-related cognitive decline. Moreover, in mice, LFS at 40 Hz has been shown to attenuate pathological hallmarks of neurodegeneration, such as beta-amyloid aggregation. However, the consequences of gamma oscillations on mental health together with the underlying mechanisms remain largely elusive. Therefore, the current study aims to investigate the behavioral outcomes of LFS at 40 Hz and 60 Hz on cognition, anxiety, and depression-like behavior. We will afterward investigate the cellular and molecular mechanisms behind its outcomes, focusing on the brain's innate immune cells, microglia, which play key roles in mental health. Thereby, adult wild-type mice are exposed to constant light control (intensity 3.9 × ^1018 photons/cm^2/s) or LFS (at the same intensity) at 40 Hz and 60 Hz for 2 hours/day for 5 days. Two hours after the last control or LFS session, mice are submitted to the open field test, novel object recognition test, and forced swim test, sequentially. Overall, we expect reduced anxiety and depression-like behavior, and improved recognition memory, especially following 40 Hz gamma LFS treatment. Understanding the effects of LFS at gamma-band frequencies on behavioral symptomatology in mice will shed light on the potential of this strategy as a novel and non-invasive therapeutic paradigm for a plethora of neuropsychiatric diseases. Moreover, this work will open new avenues for a better understanding of the neurobiological correlates underpinning behavioral changes, notably involving microglia using correlative imaging and multi-omics, in response to LFS strategies.

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Keyword: *Light flickering strategies, gamma band frequency, mood disorders, behavior, microglia*

#385 Deferiprone Promoted Myelin Repair and Functional Recovery in Demyelinated Optic Nerve

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Aim & Background: Remyelination often fails in patients with multiple sclerosis (MS), leading to chronic demyelination and axonal degeneration. Therefore, pharmacological approaches toward enhanced remyelination are highly demanded. Recently, besides its iron-chelating ability, deferiprone (DFP) exerts neuroprotective effects through various mechanisms. Here, we aimed to investigate the effects of DFP treatment on remyelination process following local demyelination.

Methods: Focal demyelination was induced by injection of lysolecithin (LPC), into the optic nerve of male C57BL/6J mice, behind the globe. The animals received daily intraperitoneal injection of DFP (10 mg/kg) or saline, twice a day, starting at 7 days post LPC injection (7 dpi). Histopathological, electrophysiological and behavioral studies were used to evaluate the outcomes. The animals were sacrificed at 14 or 21 dpi.

Results: Data analysis showed that FluoroMyelin and MBP intensity in DFP-treated group was significantly higher than the Control, suggesting enhanced remyelination at 14 ($p<0.01$) and 21 dpi ($p<0.05$). Semi-thin sections stained with toluidine blue indicated a decreased g-ratio and increased myelin thickness in DFP-treated animals compared to LPC injected group showing promoted remyelination of the demyelinated axons. At mechanistic level, DFP enhanced oligodendrogenesis by proliferation of OPCs and ameliorated gliosis during remyelination period. Our data showed that the total number of $olig2^+$ cells was significantly increased in DFP-treated group compared to Vehicle ($p<0.05$). Furthermore, the number of newly generated oligodendrocytes ($BrdU^+/olig2^+$ cells) was significantly increased in DFP group ($p<0.05$). The immunostaining against Iba1 and GFAP showed a moderate trend of microgliosis reduction and attenuated astrogliosis by DFP treatment at both time points ($p<0.05$). Furthermore, our results indicated that enhanced remyelination led to functional recovery as

evaluated by the visual evoked potential and measuring the P1 latency, which was reduced in mice treated with DFP during the remyelination phase ($p<0.01$ compared to Vehicle). To evaluate weather histological and electrophysiological improvements of visual pathway deficits led to the functional improvement in visual acuity, visual cliff and placing tests were performed. DFP treatment at remyelination phase significantly recovered the visual acuity and improved the acuity score at 14 dpi compared to vehicle group ($p<0.05$). Also, there was a significant negative correlation between P1 latency and the time in the pattern area and the placing score.

Conclusion: Even though, the exact molecular mechanisms by which DFP enhanced the myelin repair remain to be elucidated, these results raise the possibility for using DFP as a therapeutic agent for remyelination therapy in MS.

Keyword: *Multiple sclerosis, Myelin repair, Deferiprone, Oligodendrogenesis, Functional recovery*

Infection and neuroinflammation

#22 The immune response to EBV viral infection in untreated persons with multiple sclerosis and after B cell depletion

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Background: Epstein-Barr virus (EBV) infection and HLA-DRB1*15:01 are the two strongest established risk factors identified for multiple sclerosis (MS) and a synergistic effect between these risk factors has been suggested. In addition, since EBV infects mainly B cells and B cell depleting treatment (BCDT) is highly effective against active disease, it is of interest to study EBV latent infection characteristics after BCDT.

Objectives: In this project, we study EBV viral load in blood, anti-EBV antibody responses, and the phenotypic characteristics of EBV-specific T cell subsets in healthy controls (HC) and persons with MS (pwMS) before and after BCDT.

Methods: The study comprises 94 HC, 163 untreated pwMS and 172 BCD-treated pwMS, in which a subset are paired samples collected at baseline and after BCDT (n=40). Detection and quantification of EBV viral load (EBNA-1 and EBNA-LP) in DNA from whole blood was analyzed by qPCR, and IgG antibody levels against EBV (EBNA-1 and capsid antigen (CA)) and cytomegalovirus (CMV) were quantified by ELISA. Additionally, all individuals were genotyped for the presence of HLA-DRB1*15:01 and HLA-A*02:01 alleles by PCR. A subset of untreated pwMS (n=26), HCs (n=32), and paired samples (before/after BCDT, n=9), selected based on HLA-type and levels of EBV viral load, were then

assessed for EBV-specific T cell responses by flow cytometry.

Results: Similar proportion of individuals with detectable viral load was observed between untreated pwMS (52.1%) and HC (45.7%) with no differences in levels of EBNA-1 or EBNA-LP viral load. After BCDT, the viral load became undetectable in most pwMS (85.5%), and displayed a significant difference compared to both untreated pwMS and HC for both genes (p=<0.0001). IgG specific antibodies against EBNA-1 and CA remained at similar levels both before and after BCDT. However, higher levels of IgG against EBNA-1, CA, but not CMV were observed between pwMS and HC (p=<0.0001, p=<0.0001, and p=0.0702, respectively). Data on the phenotypic characteristics of EBV-specific T cells will be included in the final presentation.

Conclusion: Although anti-EBV antibody levels are higher in pwMS than HC, no difference was observed in EBV viral load. After BCDT, viral load was significantly decreased with no change in antibody levels. These data provide information on the complexity of the immune response against EBV in pwMS, also after BCDT.

Keyword: *Epstein-Barr virus, multiple sclerosis, Viral load, B cell depleting treatment*

#25 The role of age associated B cells in autoimmunity

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Age-associated B cells (ABCs) are a unique population of memory, antigen-specific B cells that exhibit distinct transcriptional and functional profiles. These cells can be characterized by their expression of T-bet, CD11c, CD11b, and lack of CD21. In females, ABCs show a disproportionate expansion with age, viral infection, and various autoimmune diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). Differential ABC phenotypes have been studied separately in females during ageing, infection, and autoimmune disease. However, since these events can occur concurrently, it is important to understand the interplay between autoimmune disease and viral infection. Previous studies have shown that murine gammaherpesvirus 68 (ghv68), an EBV murine equivalent, exacerbated experimental autoimmune encephalomyelitis (EAE) and drove a Th1-skewed condition similar to MS. This enhancement was not observed in ABC-deficient mice. In this study, we demonstrate that the ABC population expands in peripheral blood during Epstein-Barr virus (EBV) infection and MS, although the virus and autoimmune disease affect their phenotypes differently. This relationship was also investigated in mice using ghv68 and EAE. We found that splenic ABCs were expanded in mice with latent ghv68 infection, while this was observed only in female mice with EAE alone. There was no sex bias in the expansion of ABCs during ghv68-EAE. Our results provide evidence that EBV and other viral infections may prime cell subsets, such as ABCs, to facilitate autoimmune disease

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#31 Impact of SARS-CoV-2 on the neuropathogenic potential of myelin-primed Th17 cells in animal models of MS.

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). Due to sub-optimal responses to SARS-CoV-2 vaccines, MS patients are at high risk for COVID-19, a disease in which ~14% of convalescents experience neurological symptoms. Importantly, comparison of COVID-19 and MS brain autopsies showed similar neuropathology including astrocytosis, axonal damage, blood-brain barrier leakage and microglial nodules. It is critical to learn whether SARS-CoV-2-induced brain pathology augments MS disease severity or accelerates progression. As such, we wish to determine whether SARS-CoV-2 infection augments the neuropathogenic potential of myelin-primed Th17 cells in animal models of MS. We have established a working model that combines experimental autoimmune encephalomyelitis (EAE) with SARS-CoV-2 infection. Following passive EAE induction, I infect mice with a non-lethal dose of SARS-CoV-2 and assess: **(1)** clinical presentation of both EAE and SARS-CoV-2 infection using pre-established scoring systems, **(2)** CNS pathology, and **(3)** immune cell activation using flow cytometry. In a pilot experiment, SARS-CoV-2 susceptible Syrian hamsters were infected with 10^5 TCID50 of the D614G SARS-CoV-2 strain. Infected hamsters exhibited meningeal inflammation, including the accumulation of myeloid cells, like observations in humans. Next, I adoptively transferred different doses of Th17 cells derived from mice immunized with either MOG35-55 peptide or ovalbumin peptide to induce EAE in male hACE2-KI mice. During the chronic phase of disease, mice were infected with the delta variant of SARS-CoV-2 (2.5x10^6 PFU). Interestingly, hACE2-KI mice that received higher doses of MOG-primed Th17 cells experienced worsened symptoms of SARS-CoV-2 infection compared to lower doses of Th17 cells or OVA-primed Th17 cells. Unexpectedly in the reciprocal experiment, where male hACE2-KI mice received encephalogenic Th17 cells **after**

the clearance of a prior SARS-CoV-2 infection, animals with prior viral infections exhibited less severe symptoms of EAE during the chronic phase of disease compared to uninfected mice with EAE. With further work, we hope to understand the impact of SARS-CoV-2 infection on MS disease course and neuroinflammation.

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Keyword: *SARS-CoV-2, Multiple Sclerosis, Neuroinflammation, Brain, Infection*

#91 Murine Gamma-Herpesvirus 68 (yHV-68) and Canine Distemper Virus (CDV) Co-infection as a Multiple sclerosis (MS) like mice model.

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Multiple sclerosis (MS) is a neuroimmune disease caused by genetic and environmental factors. Infection with Epstein Barr virus (EBV) has an established participation in MS (1). Infection with Canine Distemper Virus (CDV) exhibits a demyelinating pathology and is considered a natural MS-like dog model (2,3). Previous studies have shown that MS patients have higher antibodies titres to CDV compared to controls (2,3). We propose a novel model for MS-like disease in mice using co-infection of Gamma-Herpesvirus 68 (yHV-68, the murine virus homologue to EBV) and CDV (4,5). Early infection of yHV-68 and establishment of viral latency may act as an immune modulator (4,5), and secondary infection with CDV may induce MS-like pathologies. Specifically, C57Bl/6 mice were infected with yHV-68, and 5-weeks later, when viral latency had established, they were infected with CDV. 5-weeks later, they were infected again with a higher dose of CDV. Mice were grouped as uninfected, yHV-68 infected, CDV infected and co-infected. The model was then validated using clinical tests, including olfactory, rotarod, facial sensibility, and vestibular stimulation (VS) tests, to evaluate early MS-like symptoms. Clinical tests were performed at baseline, and throughout infection periods. Flow cytometric analyses of the brain, spinal cord (SC) and spleen were performed to characterize immune cell modulation. Histological analyses using H&E and eriochrome cyanine were performed on the brain and SC. We found that co-infection showed a strong resemblance to MS in clinical symptoms, immunology, and histopathology. Clinical symptoms such as facial pain, olfactory disabilities, and motor deficit were observed after the second period of CDV infection. We found a diversification of VS symptoms, such as retropulsion, tumbling and freezing. Mice in the co-infected group showed higher levels of CD44, CD62L and CD69 compared to controls after the second CDV infection (7-days after in the SC, 15-days after in the brain). After the second CDV infection, co-infected mice showed elevated levels of CD8, CD4, CD19, INF-y, IL-17, FOX-P3, CD25, Rory, and Tbet in the brain

and SC compared to control groups. The γHV-68 and CDV co-infection model can contribute mechanisms by which environmental factors, such as viral infection, can relate to MS pathologies.

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#207 Persistent alterations of microglia states is measured after injury affecting the developing brain

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Background: Extreme preterm infants are exposed to multiple inflammatory stressors including perinatal cerebellar hemorrhage (CBH) and postnatal infection, two major risk factors for neurodevelopmental impairments. Microglial core properties will be assessed to further characterize the impact on microglial cells function in the pathogenesis of cerebellar injury during development.

Methodology: Mice were exposed to CBH at postnatal day 2 (P2) combined or not with early inflammation (LPS). Microglia phenotypic changes across time (P2, P3, P7 and P15) were analyzed by flow cytometry using a panel of markers. Residual phagocytosis capacity of microglial cells was analyzed using a standardized bead assay and immunostaining techniques.

Results: Our data showed that two weeks after being exposed to perinatal insults (P15), cells featuring M2 phenotypic profile are significantly decreased in mouse pups exposed to a systemic inflammatory stress alone (LPS: 10,88%, *P=0.040, n=7) or exposed to combined insults (CBH+LPS: 11,31%, *P=0.039, n=8) compared to controls (29,02 %, n=7)translating anomalies in tissue repair function. Primary cell culture of microglia exposed to insults showed a very low residual phagocytic capacity from all exposure groups (median<0.000% [0,0],****P<0,0001) compared to controls (0,512% [0,1]).

Conclusions: Perinatal insults exposure alters tissue repair and core properties of microglial cells post-injury, which translate to altered microglia proliferation and tissue remodeling responses and may create a vulnerability window during the recovery phase of injury.

Keyword: *Cerebellum, microglia, development, preterm*

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#225 Clinical and Real-World Pharmacovigilance Data of Meningococcal Infections in Eculizumab- or Ravulizumab-Treated Patients

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Background and aims: Terminal complement inhibiting therapies (C5ITs) initially approved to treat rare hematologic disorders and, more recently, rare neurologic disorders are associated with increased Neisseria meningitidis (Nm) infection risk. Robust risk mitigation measures implemented worldwide include vaccination, education materials, and patient safety cards. A pharmacovigilance analysis of exposure-adjusted incidence and mortality data for Nm infections in eculizumab- or ravulizumab-treated patients in clinical trial and real-world settings evaluated infection and mortality rates over time as exposure substantially increased.

Methods: A search of the Alexion safety database was performed for eculizumab (Mar 2007–Oct 2022) and ravulizumab (Dec 2018–June 2022) across all indications using the MedDRA High Level Term of Neisseria infection. Identified cases were reviewed to include only those associated with Nm.

Results: Cumulative clinical trial Nm infection rates for eculizumab- or ravulizumab-treated patients across 4 indications were approximately 0.30 and 0.21 cases per 100 patient-years, respectively (**Table 1**). Cumulative postmarketing reporting rates for Nm infections in eculizumab- or ravulizumab-treated patients were stable at approximately 0.24 and 0.08 cases per 100 patient-years, respectively (**Figure and Table 2**).

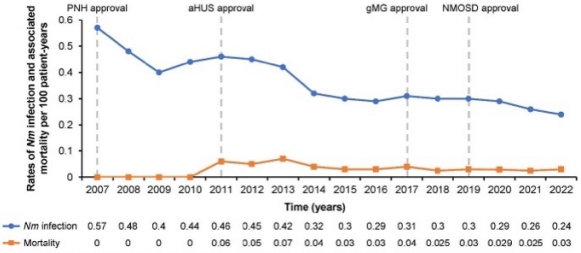
Conclusions: Although cumulative exposure to eculizumab has increased, including with the addition of rare neurologic indications, Nm infection rates have steadily decreased and mortality rates have remained stable since 2007. Comparable rates were observed in patients treated with ravulizumab. Raised infection awareness, risk mitigation strategies, and availability of additional vaccines effectively reduced the risk of Nm infections in C5IT-treated patients, underlining the importance of adhering to these measures.

Table 1. *Nm* infection and mortality rates among eculizumab- and ravulizumab-treated patients in the clinical trial setting

Treatment	Cumulative exposure, PY	*Nm* infection, rate per 100 PY	*Nm* mortality, rate per 100 PY	Total *Nm* infections	Total *Nm* fatalities
Eculizumab[1]	2,331	0.30	0	7 cases per 2,331 PY	0
Ravulizumab[2]	2,870	0.21	0.03	6 cases per 2,870 PY	1

[1]Eculizumab first approvals: PNH in 2007, aHUS in 2011, gMG in 2017 and NMOSD in 2019. Data collected between March 2007–October 2022. [2]Ravulizumab first approvals: PNH in 2018, aHUS in 2019 and gMG in 2022. Data collected between Dec 2018–June 2022.
aHUS, atypical haemolytic uraemic syndrome; gMG, generalized myasthenia gravis; *Nm*, *Neisseria meningitidis*; NMOSD, neuromyelitis optica spectrum disorder; PNH, paroxysmal nocturnal haemoglobinuria; PY, patient-years

Figure. Rates of *Nm* infection and associated mortality for eculizumab per 100 patient-years from 2007 to 2022 in the real-world setting



Data are expressed as cumulative rate per 100 patient-years and are inclusive of PNH, aHUS, gMG and NMOSD (indications approved in March 2007, September 2011, December 2017 and June 2019, respectively). Ravulizumab data are not shown owing to low number of cases ($n = 6$) as of data cut-off.
aHUS, atypical haemolytic uraemic syndrome; gMG, generalized myasthenia gravis; *Nm*, *Neisseria meningitidis*; NMOSD, neuromyelitis optica spectrum disorder; PNH, paroxysmal nocturnal haemoglobinuria.

Table 2. *Nm* infection and mortality rates among eculizumab- and ravulizumab-treated patients in the real-world setting

Treatment	Cumulative exposure, PY	*Nm* infection, rate per 100 PY	*Nm* mortality, rate per 100 PY	Total *Nm* infections	Total *Nm* fatalities
Eculizumab[1]	78,416	0.24	0.03	191 cases per 78,416 PY	20
Ravulizumab[2]	7,533	0.08	0.02	6 cases per 7,533 PY	1

[1]Eculizumab first approvals: PNH in 2007, aHUS in 2011, gMG in 2017 and NMOSD in 2019. Data collected between March 2007–October 2022. [2]Ravulizumab first approvals: PNH in 2018, aHUS in 2019 and gMG in 2022. Data collected between Dec 2018–June 2022
aHUS, atypical haemolytic uraemic syndrome; gMG, generalized myasthenia gravis, *Nm*, *Neisseria meningitidis*; NMOSD, neuromyelitis optica spectrum disorder; PNH, paroxysmal nocturnal haemoglobinuria; PY, patient-years

#235 Clinical and radiological characteristics of COVID-19-associated myelitis

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Background: The emergence of acute respiratory syndrome coronavirus 2 and coronavirus disease 2019 (COVID-19) pandemic has led to an increased recognition of various neurological complications associated with COVID-19. Herein, we report a study on the clinical and radiological characteristics of COVID-19-associated myelitis following COVID-19 infection or immunization.

Methods: We examined the medical records of the myelitis patients admitted to Asan Medical Center from January 2020 to March 2023. We defined post-infectious COVID-19 transverse myelitis (PITM) as myelitis occurring within 3 months of the onset of COVID-19 symptoms, and post-vaccination transverse myelitis (PVTM) as myelitis occurring within 3 months of COVID-19 vaccination. We also defined idiopathic transverse myelitis (ITM) as myelitis whose etiology remains unknown despite a comprehensive diagnostic work-up. Then, we compared the clinical and radiological characteristics of the patients with PITM, PVTM, and ITM.

Results: Patients with PITM (n=13), PVTM (n=12), and ITM (n=27) had mean ages of 46.2, 55.3, and 47.6, respectively, and approximately 60% of the patients were male. The modified Rankin scale (mRS) on the first day of hospitalization was higher in patients with PITM (3.9) compared to those with ITM (2.2, p=0.001) and PVTM (2.3, p=0.002). Among patients with PITM, a higher mRS was noted with short latency group (<1 week; 5.0), compared to intermediate (1 week to 1 month; 4.3, p= 0.250) and long latency group (1 month to 3 months; 3.3, p=0.036). The proportion of patients with good outcome (mRS ≤1) at 6-months follow-up was lower in PITM (23.1% vs. 50% for PVTM, 70.3% for ITM, p=0.018). PITM group demonstrated longer spinal cord involvement (11.5 segments) than PVTM (5.3 segments, p=0.035), and ITM (3.5 segments, p=0.002), with multifocal lesions on axial image (61.5%, p=0.006). In addition, bilateral corticospinal tract involvement extending to the posterior limb of internal capsule was observed in only PITM (n=3), and magnetic resonance imaging (MRI)-negative myelitis was observed in both PITM (n=1) and PVTM (n=1) groups.

Conclusions: PITM was associated with longitudinally extensive and multifocal spinal cord involvement, a more severe clinical deficit at onset, and a poor clinical outcome. In addition, bilateral corticospinal tract involvement and MRI-negative myelitis may be distinctive radiologic characteristics of COVID-19-associated myelitis.

#240 Rapamycin as a new adjunctive treatment in murine pneumococcal meningitis

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New treatments for pneumococcal meningitis are needed to improve outcome. We previously showed that V-akt murine thymoma viral oncogene homolog 3 (AKT3) influenced outcome in meningitis and hypothesized that this is mediated through regulation of mammalian target of rapamycin (mTOR). mTOR is a central regulator of cellular metabolism that shapes

immune effector responses. In animal models, mTOR inhibition can modulate the immune system and improve outcome in sepsis and influenza. Here we test rapamycin as adjunctive treatment in a murine model of pneumococcal meningitis.

C57BL/6N mice of both sexes aged 8-12 weeks were intracisternally inoculated with Streptococcus pneumoniae (serotype 2). Mice were treated with ceftriaxone (100 mg/kg) at 20 and 44 hours post inoculation (hpi). Animals were randomized into a treatment group receiving 5 mg/kg of rapamycin, and a control group receiving vehicle, directly before inoculation and concomitant with antibiotic treatment in an investigator-blinded fashion. In clinical severity experiments mice were assessed with a clinical severity score for 72 hours or taken out of the experiment when they reached a humane endpoint. In time point experiments mice were terminated at 6 or 24 hpi. Evaluation was performed of leukocyte infiltration in the CSF, gene expression in brain and spleen, cytokine levels in the blood and bacterial outgrowth in all compartments.

Clinical scores were not significantly different between treated mice and controls. Median time until reaching a humane endpoint was 36 hpi for treated mice and 42 hpi for controls (ns). Rapacymin treatment upregulated gene expression of Akt3 at 24 hpi in spleen (fold change 2.0, P = 0.01) and slightly in the brain (fold change 1.1, P = 0.03). Treatment increased cerebrospinal fluid leukocyte count at 6 hpi (6*10^5 cells/ul vs 3*10^5 cells/ul), although not significantly (P = 0.05). Levels of IL-12p70 were elevated in the blood of treated mice at 24 hpi (median 216 pg/ml) compared to controls (median 6.7 pg/ml, P < 0.01). Gene expression of IL-10 and IL-12p70 was equal between groups. Bacterial outgrowth in all compartments was equal across treatment groups for both time points.

Rapamycin treatment did not influence clinical severity in murine pneumococcal meningitis. Treatment modulated AKT3 gene expression and increased inflammation at both, early and late time, points. Rapamycin treatment is not beneficial as adjunctive treatment in murine pneumococcal meningitis.

Figure 1: Clinical scores (mean+/-SD) comparing treated and control mice. Mice are scored as 15 from the moment they reach a humane endpoint.

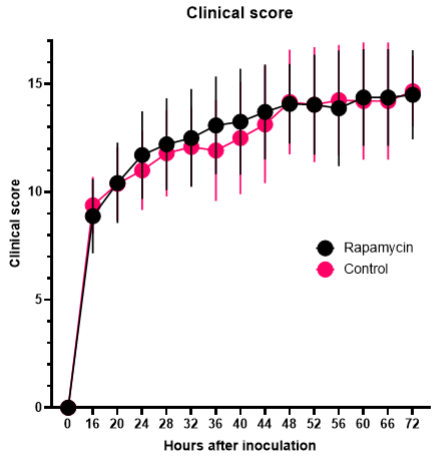


Figure 2: AKT3 gene expression in the brain and spleen at 24 hpi. Expression is shown as fold change compared to expression in the vehicle group.

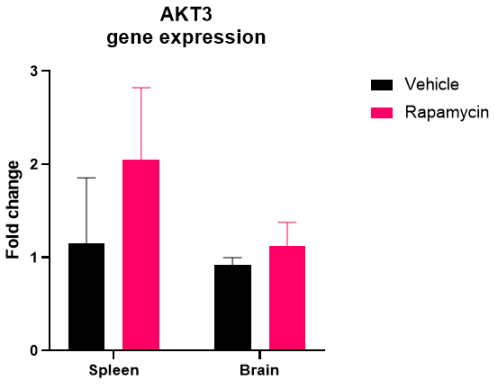
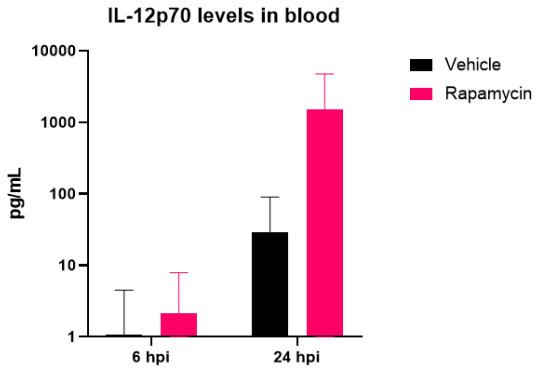


Figure 3: Levels of IL-12p70 in the blood at 6 hours and 24 hours post infection.



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Keyword: *bacterial meningitis, rapamycin, adjunctive treatment, Streptococcus pneumoniae, experimental meningitis*

#248 Functional analysis of UBE2U in pneumococcal meningitis

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A genome-wide association study performed by our group identified a Single Nucleotide Polymorphism located in the intronic region of the ubiquitin conjugation enzyme E2 U (UBE2U) gene that was associated with unfavourable outcome in patients with bacterial meningitis (MAF = 0.43; odds ratio (OR) = 1.63; p = 2.0×10^-8). UBE2U is a component of the ubiquitination pathway, a system that regulates several immune functions and is often impaired by bacterial pathogens in order to evade host immune responses. Here, we studied the role of UBE2U in pneumococcal meningitis.

Immunohistochemistry for UBE2U was performed on hippocampal slides of patients with pneumococcal meningitis, and subsequently compared to control patients that died from causes unrelated to cerebral infection. Stained sections were scored on UBE2U expression by a neuropathologist blinded for the patient status. As an in vitro model for neurons, differentiated neuroblastoma cells (SH-SY5Y) were stimulated with 1 ug/mL lipoteichoic acid (LTA) or lipopolysaccharides (LPS) for 24 hours, after which expression of UBE2U was assessed by immunocytochemistry and Western blotting.

UBE2U protein expression was decreased in hippocampal neurons and glia cells in patients with pneumococcal meningitis compared to control patients. Because the strongest expression was observed in neuronal cells we used a neuroblastoma cell line to evaluate UBE2U expression after stimulation. Immunocytochemistry showed that LTA- and LPS-stimulated SH-SY5Y cells had increased expression and localization of UBE2U around the

nucleus in comparison to unstimulated cells. Western blot analysis did not show an upregulation of UBE2U in LTA-stimulated cells after 24 hours.

While UBE2U expression was decreased in the hippocampus of patients with pneumococcal meningitis, our in vitro research suggests UBE2U levels to be elevated upon stimulation with bacterial components LTA and LPS. This indicates that UBE2U-dependent ubiquitination is important during pneumococcal meningitis and might be targeted by Streptococcus pneumoniae in order to disrupt host immunity.

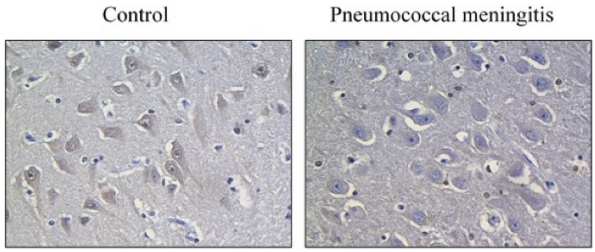

Figure 1. UBE2U expression is decreased in hippocampal neurons in patients with pneumococcal meningitis.

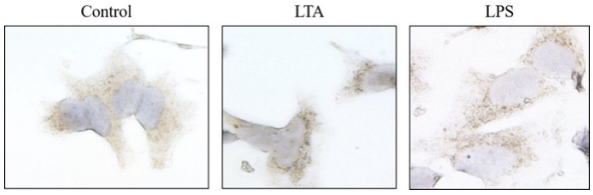

Figure 2. Stimulation with LTA and LPS increases localization of UBE2U around the nucleus

#280 Tumor Necrosis Factor Alpha Inhibitors as First-Line Steroid Sparing Therapy for Neurosarcoidosis: A Case Series

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Background: Sarcoidosis is a systemic inflammatory disease characterized by non-necrotizing granuloma formation which can variably involve multiple organ systems including the nervous system in neurosarcoidosis. While several case series have demonstrated favorable outcomes in neurosarcoidosis patients treated with tumor necrosis factor alpha (TNF-alpha) inhibitors, there is currently no consensus on preferred steroid-sparing treatments for neurosarcoidosis. Aim: To describe the clinical course of patients treated with TNF-alpha inhibitors as first-line steroid sparing therapy. Methods: This retrospective case series spanning from 2010-2022 consisted of 8 adults with probable or definite neurosarcoidosis based on Neurosarcoid Consortium Consensus Group criteria. Patients were seen at the University of Vermont rheumatology or neurology clinics, had at least one follow-up visit, and received TNF-alpha inhibitor therapy as first-line steroid sparing therapy. Data collected through chart review included demographics, disease characteristics, treatment course, and treatment response. Results: Of the 8 neurosarcoidosis patients, most were male (n=6) and the median age at diagnosis was 55, interquartile range (IQR) 23 (42-65). Half were definite diagnoses (n=4) confirmed by brain biopsy. The most commonly involved neurologic tissues were parenchymal (n=7), meningeal (n=4), and spinal (n=3). Outside of neurologic involvement, most patients had lung involvement (n=6) and some had musculoskeletal (n=2) involvement. Based on available cerebrospinal fluid studies, most had elevated protein (n=6) and white blood cell counts (n=6), with negative results for angiotensin converting enzyme (n=7), IgG (n=8), and oligoclonal bands (n=8). Most patients were

started on infliximab (n=7), with TNF-alpha inhibitors initiated a median of 5.7 months after neurosarcoidosis diagnosis, IQR 8.2 (3.4-11.6). Most patients were co-treated with methotrexate (n=7). Most patients were able to wean to <5 mg of prednisone (n=6) in a median of 2.6 months, IQR 1.3 (2.1-3.5). Most patients had one or fewer relapses (n=7) and achieved partial or complete clinical remission (n=7). A few patients had adverse reactions including pulmonary infections (n=3) and skin infections (n=1). One patient died 12 years after diagnosis. Conclusion: This study provides further support for the use of TNF-alpha inhibitors as first-line steroid sparing therapy in neurosarcoidosis. Among the 8 neurosarcoidosis patients treated with TNF-alpha inhibitors, most were able to wean from steroids within 6 months and achieved partial or complete clinical remission.

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Keyword: *Neurosarcoidosis, Infliximab, Tumor Necrosis Factor Inhibitors*

#331 Distinct populations of viral-specific resident memory T cells trigger adaptive and innate immune activation in the brain

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Resident memory T cells are a unique subset of memory T cells that persist within tissues throughout the body, including the brain, optimally positioned to exert local immune activation. Broadly, T cells in the CNS are implicated in a range of neurologic disorders, can protect against reinfection, and even participate in normal CNS homeostasis. Despite this, there remain significant gaps in knowledge about how T cells, specifically resident memory T cells (TRM), are regulated and function in the unique CNS environment. Here, we leverage a mouse model of acute viral infection to dissect the diversity of brain TRM and their impact on local immune environment. Through single cell RNA sequencing we find unique brain TRM populations with transcriptional signatures predictive of distinct functions. Interestingly, we find that the route of initial infection shapes population diversity of established brain TRM months after the infection has cleared. We identify cytolytic subsets unique to mice infected intranasally when compared to those infected systemically, likely indicating a role for local antigen encounter in shaping brain TRM populations. Additionally, we report a shared subset of TRM persisting in the brains of mice independent of infection route, establishing a

core brain TRM transcriptional profile. Our data confirmed previous findings that brain TRM express markers associated with T cell exhaustion, such as PD-1. Despite this inhibitory profile, we show that intracranial delivery of cognate viral peptide led to robust bTRM reactivation and initiated a cascade of immune activation and accumulation within the brain, including rapid activation of microglia, NK cells and T cells, DC maturation, and infiltration of macrophages and monocyte derived DCs. In the presence of PD-L1 blockade or genetic deletion of PD-1, despite observing higher effector molecule production from reactivated bTRM, we found no apparent difference in downstream immune activation in the brain nor enhanced brain TRM mediated protection from reinfection. Our studies illuminate a previously unappreciated role for local antigen in shaping brain TRM heterogeneity, likely reflecting diverse functions, and demonstrate the capacity for TRM alarm functions to potently activate local immunity. Collectively, these data provide a foundational knowledge base upon which to contextualize the role of TRM in pathogenic settings and may guide therapies to target these cells.

#349 Dysregulated neurogenesis and impaired cognitive function following murine coronavirus infection

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Adult hippocampal neurogenesis has been implicated in cognitive processes. While there is no direct evidence for the invasion of coronavirus (CoVs), including SARS-CoV-2, into the hippocampal region of the brain, considerable proportions of individuals who had COVID-19 experience “brain fog”, a term applied to a variety of cognitive symptoms including memory disorders, lasting several months after the initial infection. We therefore explored the consequences of neurotropic mouse CoV infection on cell proliferation, neurogenesis and cognitive performance after virus control. We employed the mouse hepatitis virus (MHV), MHV-JHM variant (JHMV), as a well-established viral model for acute encephalomyelitis followed by demyelination during the chronic phase. Following intracranial infection, clinical symptoms peaked at day 14 post-infection (pi) and resolved by day 21 pi, with viral controlled by T cell responses, particularly through interferon-gamma (IFNg) production. However, it remains unclear whether these mice experience impaired cognitive function following viral control. To address this, we used Ki67 and doublecortin (DCX) immunohistochemistry to evaluate cell proliferation and neurogenesis in the dentate gyrus (DG) of the hippocampus over time. Our findings revealed a significant increase in the number of Ki67 positive cells in the subgranular zone (SGZ) of the DG in infected mice at day 21 pi compared to age-matched control mice. However, by day 42 pi, the Ki67 positive cells exhibited a trend toward being lower than those in control mice. Increased DCX immunoreactivity in the SGZ of JHMV-infected mice indicated enhanced neurogenesis. Interestingly, irregular staining patterns of DCX-positive neuroblasts or immature granule cells in the granule cell layer suggested dysregulated migration and maturation of new-born neurons in virus-infected mice. The novel object recognition (NOR) test was utilized to assess cognition, particularly recognition memory after virus control. The NOR test revealed impaired long-term memory in JHMV-infected mice compared to control mice. Notably, JHMV-infected mice exhibited a trend towards reduced anxiety levels in a stressful environment relative to control mice. These findings support that CoV infection

may dysregulate hippocampal neurogenesis, impair cognitive function, and influence normal anxiety responses even in the absence of direct hippocampal infection and after viral control. Our study supports the hypothesis that virus-induced alterations in the hippocampus contribute to cognitive deficits and adds to findings of impaired hippocampal neurogenesis in a SARS CoV2 murine infection model. Further research is warranted to elucidate the underlying mechanisms for development of potential therapeutic interventions.

#362 Multicentric evaluation of a specific intrathecal anti-Treponema pallidum IgG index as a diagnostic biomarker of neurosyphilis: results from a retro-prospective case-control study

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Importance: The diagnosis of neurosyphilis (NS) lacks a true "gold standard" which makes the diagnosis challenging while the consequences of a misdiagnosis are potentially severe.

Objective: The aim of this study was to evaluate the diagnostic performance of measuring an antibody index (AI) for intrathecal synthesis of specific anti-Treponema pallidum (Tp) IgG for the diagnosis of NS.

Design, setting, and participants: We evaluated an AI for intrathecal synthesis of specific anti-Tp IgG on paired cerebrospinal fluid (CSF)-serum samples collected between 2007 and 2022 from patients suspected of NS, in Switzerland. Two definitions for NS were used: NS1 included patients with suspicion of NS presenting symptoms suggestive of central nervous system (CNS) involvement, and positive TPHA/TPPA serology and CSF-TPHA/TPPA≥320, and either CSF-leucocytes >5 cells/mm^3 and/or CSF-protein >0,45g/l and/or a reactive CSF-VDRL/RPR test. NS2 included patients with suspicion of NS presenting acute ocular and/or otologic symptoms, and positive TPHA/TPPA serology, and a favorable response to NS treatment. Controls were patients diagnosed with any other CNS pathologies and with positive TPHA/TPPA serology.

Main Outcomes and Measures: CSF parameters were analyzed and anti-Tp IgG were measured simultaneously in serum and CSF. AI was calculated according to Reiber diagram. We estimated the AI test area under the ROC curve, its sensitivity/specificity, and positive and negative predictive values using plausible NS prevalence reflecting routine data.

Results: The study included 71 NS (43 NS1 and 28 NS2) and 110 controls. With a threshold of ≥1.7, sensitivity and specificity of the specific AI test were 90.7% (IC 77.7-97.4) and 100% (IC 96.7-100.0) respectively for NS1 and 14.3% (IC 4-32.7) and 100% (IC 96.7-100.0) for NS2. In patients suspected of NS with a CNS involvement (NS1 group), where NS had a prevalence of 28%, neurosyphilis could be confirmed by the positivity of this specific AI.

Conclusion and Relevance: The measure of an intrathecal synthesis index of specific anti-Tp IgG in patients with inflammatory signs in CSF appears to be a reliable diagnostic test to assess a neurosyphilis. However, in otic or ocular syphilis, presenting few or no abnormalities of CSF, the AI alone is not sufficient to rule-out the diagnosis of neurosyphilis.

#366 CSF features in Treponema, Borrelia and HIV infections with CNS involvement and intrathecal synthesis

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Background: The detection of intrathecal oligoclonal immunoglobulin G (IgG) synthesis is beneficial in supporting the clinical diagnosis of multiple sclerosis (McDonald criteria, 2017). It also serves as a diagnostic tool to determine central nervous system (CNS) involvement in specific autoimmune or infectious disorders. Utilizing the isoelectric focusing technique, two oligoclonal band (OCB) patterns suggest intrathecal synthesis of oligoclonal IgG: type 2, characterized by the presence of OCBs exclusively in CSF, and type 3, characterized by OCBs in both CSF and serum, with additional bands unique to the CSF. This study aimed to compare three infectious diseases that display intrathecal synthesis in terms of OCB patterns and CSF features: two infections caused by spirochete bacteria, such as neuroborreliosis (Borrelia burgdorferi) and neurosyphilis (Treponema pallidum) (whether co-infected with HIV or not), and neuro-HIV.

Methods: CSF cytological examination consisted of counting WBC (cells/mm^3) and evaluating the proportion of different cell types. The blood-brain-barrier (BBB) integrity was evaluated according to Reiber method[1].Intrathecal IgG production was qualitatively detected by the gold standard isoelectric focusing (IEF) method[2] according to the instructions of the Hydrasis apparatus supplier (Sebia, Evry, France) and using an IgG-specific antibody staining (Sebia). CSF and serum were analyzed on the same gel, and the presence of at least two IgG bands refers to an OCB-positive sample.

Results: Neurosyphilis (NS) HIV coinfected or not, neuroborreliosis and neuroHIV patients frequently showed an intrathecal synthesis (63%, 83%, 70% and 100%, respectively) and increased IgG index (25%, 75%, 50 % and 93%, respectively) (Table 1). The number of OCB patterns 2 (P2) and 3 (P3) did not significantly differ among these diseases. CSF cellularity was characterized by a leucorachia particularly increased in neuroborreliosis compared to NS and neuroHIV. The Qalb calculation revealed a frequent BBB disruption especially in neuroborreliosis patients (90%) compared to NS without HIV coinfection (25%). CSF protein level was also more increased in neuroborreliosis compared to NS, neuro-HIV and co-infected patients. The number of CSF-OCB was similar for these diseases whatever the OCB pattern. In case of pattern 3, the number of serum-OCB was extremely increased in NS co-infected with HIV in comparison with NS HIV negative or neuro-HIV.

Table1. CSF features of NeuroBorreliosis, NeuroHIV, Neurosyphilis co-infected HIV or not.

	NeuroBorreliosis (n=10)	Neurosyphilis HIV- (n=12)	NeuroHIV (n=14)	Neurosyphilis HIV+ (n=8)
Presence of OCB (%)	70	83	100	63
IgG Index ≥0.7	5/10 (50%)	9/12 (75%)	13/14 (93%)	2/8 (25%)
P2, n (%)	3 (43%)	5 (50%)	6 (43%)	1 (25%)
P3, n (%)	4 (57%)	5 (50 %)	8 (57%)	4 (75%)
CSF WBC (cells/mm^3) median (p25-p75)	92 (5.75-297)	6.5 (1-30.25)	2 (1-7)	11.5 (1.25-33.25)
Qalb > Qlim	9/10 (90%)	3/12 (25%)	8/14 (57%)	5/8 (63%)
CSF protein (g/L) median (p25-p75)	0.91 (0.69-2.57)	0.48 (0.40-0.59)	0.55 (0.42-0.71)	0.69 (0.49-1.02)
CSF plasma cells (%)	7/10 (70%)	6/11 (55%)	5/14 (36%)	5/8 (62%)
CSF-OCB number median (p25-p75)	18 (13-23)	16 (13-21)	20 (16-23)	20 (16-25)
P3 serum-OCB number median (p25-p75)	4 (2-7)	5 (3-11)	6 (5-8)	14 (7-17)

OCB: oligoclonal IgG bands, P2: OCB type2, P3: OCB type 3, WBC: white blood cells, Qalb: albumine quotient (CSF albumin/serum albumin), Qlim:limits of the albumin-quotient calculated for age corresponding to (4+age/15)*10^{-3}

Conclusion: Despite the limited number of patients, the results of this study demonstrated that: 1) spirochetal infections present distinct CSF immune reactions; 2) neuroborreliosis exhibits a more acute inflammatory burst compared to NS and neuroHIV; 3) CSF characteristics of co-infection suggest the additive effects of both NS and neuroHIV rather than a predominant result of one of these diseases.

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Schibler M, Deffert C. 2020. Cerebrospinal fluid features in SARS-CoV-2 RT-PCR positive patients. Clin Infect Dis 73:e3102–e3105. https://doi.org/10.1093/cid/ciaa1165.

Keyword: *intrathecal synthesis, neurosyphilis, neuroborreliosis, neuroHIV*

#371 Single centre analysis of paediatrics febrile convulsion with COVID-19 and other various viral infections

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Introduction) This study analysed the trend of febrile convulsions with various virus infections, including COVID-19, from January to December 2022.

Methods) We retrospectively analyzed the medical chart review of 204 pediatric patients who visited Gyeongsang National University Changwon Hospital due to a chief complaint of febrile convulsion from January to December 2022. The clinical characteristics include sex, age, past medical history and seizure semiology. We also reviewed all laboratory testing performed when patients visited our emergency centre.

Results) Among 2356 patients diagnosed with COVID-19, 808 (34.3%) patients were under 19-year-old age, and 256 patients complained of seizures with fever. We collected available data from 204 patients, and 48 (5.9%) patients were febrile convulsions with COVID-19. In addition, 32 patients (66.7%) were boys, and the median age was 2.46 (3.97±3.00 years). 4 (8.3%) patients had a family history of febrile convulsion, and 11 (22.9%) patients had previous febrile convulsion history. 7 (14.6%) patients presented a complex type of febrile convulsion, and 2 patients were injected with intravenous anti-seizure medication. Most patients present generalized seizure (97.9%), and the median seizure duration was 3 minutes (5.45±9.37 minutes).
The second most prevalent virus infection was rhinovirus, 41 (20.1%), and the third one was parainfluenza, 24(11.8%). The co-infection was defined as infection with more than two virus types in 31 (15.2%) patients. COVID-19 infection was observed predominantly from March to August, and the other virus infection trend was increased since April, which relieved the policy of social quarantine and popped up increasingly during summer vacancy. The population under 3-year-old age was predominantly observed in all infectious groups, and the population over 8-year-old age was observed in the group with SARS-CoV-2.
We analyzed the serologic markers, and leukocytosis was prominently observed in groups detected enterovirus, rhinovirus, SARS-CoV-2 and co-infection with statistical significance. The metapneumovirus group presented increased several inflammatory markers, including IL-6, CRP, and ESR, without statistical significance. In the group with co-infection, serum ESR level was increased 1.75 times compared to the group with a single viral infection. We divided the two groups by increased cytokine, IL-6, and the group with increased IL-6 presented prolonged seizure over 5 minutes compared to the group without increased IL-6 with statistical significance.

Conclusion) This study revealed that various viral infections induced febrile convulsion in pediatric populations affected by COVID-19 were observed during 2022. In addition, the various inflammatory markers and newly issued cytokine levels could affect the seizure profile in pediatric febrile convulsions.

Keyword: *COVID-19, febrile convulsion, pediatric seizure, Interleukin-6*

#388 The central nervous system's proteogenomic and spatial imprint upon systemic viral infections with SARS-CoV-2

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In COVID-19 neurological alterations are noticed during systemic viral infection. Various pathophysiological mechanisms on the central nervous system (CNS) have been suggested in the past years, including the viral neurotropism hypothesis. Nevertheless, neurological complications can also occur independent of neurotropism and at different stages of the disease and may be persistent.

Previous autopsy studies of the CNS from patients with severe COVID-19 show infiltration of macrophages and T lymphocytes, especially in the perivascular regions as well as pronounced microglial activation, but without signs of viral encephalitis.

However, there is an ongoing debate about long-term changes and cytotoxic effects in the CNS due to the systemic inflammation.

Here, we show the brain-specific host response during and after COVID-19 in the brainstem and identify region specific alterations in vagal nerve nuclei. We profile single-nucleus transcriptomes and proteomes of brainstem tissue from deceased COVID-19 patients who underwent rapid autopsy. We detect a disease phase-dependent inflammatory type-I interferon response in acute COVID-19 cases similar to other organs not directly infected and brain specific alterations as transcriptional alteration of myelinating oligodendrocytes. Integrating single-nucleus RNA sequencing and spatial transcriptomics, we could localize the neuronal alterations to vagal nerve nuclei independent of the localisation of the general response seen e.g. at the blood brain barrier.

Our results indicate that even without persistence of SARS-CoV-2 in the CNS, the tissue activates reactive mechanisms, which could cause functional disturbances that may explain the neurological complications of COVID-19 such as cardiac dysfunction, triggered by strong systemic type-I IFN signatures in the periphery.

#389 Pegivirus encephalitis - myth or truth?

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Viral infections of the central nervous system (CNS) can cause severe and sometimes fatal encephalitides, and often have a tropism for distinct brain regions, such as the temporal lobe in herpes simplex virus type 1 encephalitis. Human Pegivirus (HPgV) is a lymphotrophic virus, which has been detected in the cerebrospinal fluid (CSF) and serum of patients with encephalitis/myelitis of unknown etiology in single case studies in the past, but its exact pathology is still unknown.

Here, we report on a series of three patients in whom pegivirus RNA was detected in the CSF and of whom two died at different timepoints after onset of encephalitis. We present the clinical, radiological, virological and neuropathological characteristics. A common and very peculiar pattern of MRI abnormalities in all patients included bilateral symmetric affection of the pyramidal tracts, most prominently in the medulla oblongata, as well as of the optical tracts.

Detection of pegivirus RNA in the CSF/blood samples using RT-qPCR. We targeted the 5' UTR region of the HPgV genome. Oligonucleotides were designed based on an alignment of available pegivirus sequences. Evaluation of clinical data and cranial and spinal MRIs. Brain autopsy and neuropathological examination has been performed on two patients.

Brain and spinal MRI revealed hyperintensities in T2/DWO-weighted images along the whole pyramidal tract with main focus on the medulla oblongata, pons and mesencephalon, and internal capsule in all patients. In these regions pegivirus RNA has been detected in the tissue samples post mortem of the two fatal cases. CSF revealed pleocytosis. All patients showed alterations of the immune system.

Brain autopsy revealed lesions that corresponded to those on MRI. In the lesions, the tissue was softened resembling a subacute brain infarction. Histologically, the tissue showed time dependent increasing macrophage infiltrations with lesion age together with destruction of myelin and axons. Bizarr (tumor like) astrocytes could be detected, sometimes with nuclear inclusions. Furthermore, we detected intraparenchymal infiltrations of CD8+ T-cells and a strong upregulation of HLA-DR throughout the whole tissue.

Symmetrical bilateral affection of the pyramidal tracts in the medulla oblongata should elicit testing for pegivirus RNA in the CSF. This independent case series supports previous findings and again raises the question of HPgV causing CNS infection in immunocompromised individuals. It is still not clear which host or viral factors determine a possible neurotropism leading to encephalitis as pegivirus is present in 1 to 5% of the population.

#394 Impact of SARS-CoV2 Infection on Microglia in the Prefrontal Cortex and Hippocampus

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Infection with the novel coronavirus, SARS-CoV-2, and its associated illness, COVID-19, acutely, as well as long-COVID, are associated with a number of neurological symptoms. These range in severity, but can include anosmia, headache and

"brain fog"—an impairment of attention, concentration, memory and executive function—but also, hemorrhage, stroke and encephalitis. Therefore, understanding the neural alterations following COVID-19 is important to understand how these symptoms occur. One cell type implicated in these neurological symptoms are microglia, which are the resident innate immune cells of the central nervous system. In response to respiratory COVID, peripheral inflammation ("cytokine storm") can lead to neuroinflammation ("mirror inflammation") and microglial reactivity. This reactivity is thought to contribute to altered functions of other cells in the central nervous system, such as astrocytes and oligodendrocytes, as well as alterations in synapses, leading to aberrant brain function.

In this work, we will examine the impact of SARS-CoV-2 infection on microglia density, distribution and morphology in the prefrontal cortex and dorsal and ventral hippocampus, which are brain regions important for some of the cognitive symptoms ("brain fog") observed with COVID-19. In this work, male and female Syrian hamsters are infected with SARS-CoV-2 and examined 1-, 3- and 7-days post-infection, to investigate the acute phase of the disease. Following extraction and fixation, brains will be stained for antibodies against IBA1 (a marker of microglia and macrophages), and their density and distribution, and morphology, will be examined using epifluorescence and confocal imaging, respectively. We will follow up with ultrastructure analysis of microglia using scanning electron microscopy.

This work will provide additional information about the impact of SARS-CoV-2 on microglia during the acute infection phase. We will provide details as to alterations in microglia number and morphology, as well as insight into their interactions with other parenchymal elements at the ultrastructure level using electron microscopy. We hope that this work can contribute to the understanding of the manifestation of COVID-19 neurological symptoms.

Keyword: *microglia, covid, sars-cov-2, infection*

#396 Neural mechanisms of acupuncture for peripheral facial nerve palsy: A protocol for systematic review and meta-analysis

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Peripheral facial nerve palsy (PFNP) is a cranial neuropathy that occurs when the seventh facial nerve is damaged. PFNP seriously affects patients' quality of life, and approximately 30% of patients suffer from sequelae, such as unrecovered palsy, synkinesis, facial muscle contracture, and facial spasm. Many studies have confirmed the effectiveness of acupuncture for the treatment of PFNP. However, the specific mechanism remains unclear and needs to be further explored. Therefore, the purpose of this systematic review is to investigate the neural mechanisms underlying acupuncture treatment for PFNP using neuroimaging methods.

We will search all published studies from inception to March 2023 using the following databases: MEDLINE, Cochrane Library, EMBASE, CNKI, KMBASE, KISS, ScienceON, and OASIS. All clinical studies evaluating the effectiveness of acupuncture for treating PFNP using functional neuroimaging will be selected without language restrictions. Two reviewers will independently conduct the study selection, data extraction, and risk of bias assessment, according to a

predetermined protocol. The outcomes, including the types of functional neuroimaging techniques, brain function alterations, and clinical outcomes, such as the House-Brackmann scale and Sunnybrook Facial Grading System, will also be analyzed. Coordinate-based meta-analysis and subgroup analyses will be performed if possible.

This study will analyze the effect of acupuncture on brain activity alterations and clinical improvement in patients with PFNP using functional neuroimaging. This study will provide a comprehensive summary and help elucidate the neural mechanisms of acupuncture treatment for PFNP.

Influence of diet and microbiota on neuroinflammation

#29 Role of the aging gut microbiome in modulating murine neuroinflammation

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Multiple sclerosis (MS) is an autoinflammatory disorder of the central nervous system (CNS), wherein aberrant immune activation results in stripping of myelin and axonal damage. Microglia are known to be activated in MS and are thought to respond to infiltration of peripheral immune cells and contribute to CNS damage. Risk of developing MS and trajectory of disease progression are closely linked with environmental factors and age, respectively. The gut microbiome of MS patients diverges from that of healthy individuals, and gut microbiota from MS patients has been shown to promote disease when transplanted into mouse models. Furthermore, the onset of progression in MS patients occurs at a relatively consistent age. Similarly, chronological aging is also correlated with shifts in gut microbiota that tend to promote inflammation. Therefore, it is possible that an aged gut microbiome may promote microglia activation, thereby contributing to disease progression. The fecal microbiota transplant (FMT) model allows the introduction of human fecal bacterial populations into antibiotic-treated or germ-free mice. Demyelination is subsequently induced by adoptive transfer of encephalitogenic T cells from proteolipid protein 139-151 ($PLP_{139-151}$)-immunized donor mice. Although mouse-to-mouse FMT fails to modulate disease, young mice given an aged human FMT partially recapitulate experimental autoimmune encephalomyelitis (EAE) disease course exhibited by aged mice, and CNS microglia/macrophage pathology closely resembles that of EAE in an aged brain. Subsequent work will closely examine microglia phenotype and function, and changes in microglia induced by an aged gut microbiome. Metabolomic analyses will guide mechanistic investigation on gut-brain communication in neuroinflammation. Preliminary unbiased analyses in young or aged FMT recipient mice suggests differing metabolomic profiles dependent on age of FMT donor. Indole-3-propionic acid (IPA) was found to be differentially abundant in the sera and brains of young and aged FMT mice. Early evidence suggests that IPA may play a role in modulating microglial activation during neuroinflammation. This work may provide a novel avenue for developing a therapeutic approach for targeting disease progression.

#36 The role of the gut microbiota in the onset of progressive experimental allergic encephalomyelitis

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Multiple sclerosis (MS) is an autoimmune disease characterized by neuroinflammation, demyelination and neurodegeneration. Relapsing-remitting MS (RRMS) is the most common subtype, marked by periods of new or worsened symptoms with intervals of remission in between. Despite treatment, approximately 50% of RRMS patients will develop progressive MS within 10–15 years. In addition to RRMS, about 15% of MS patients are diagnosed with progressive MS from onset. SPMS and PPMS are associated with serious physical disability and poor quality of life and are unfortunately refractory to current approved therapies. Therefore, preventing or delaying the onset of progressive MS is critical for ameliorating disability in the disease. Our goal is to identify the mechanisms that lead to progressive MS. The gut microbiome significantly influences MS disease and interestingly, the gut microbiota is altered with aging. This is noteworthy since age is the only factor to date that correlates with progression in MS. The objective of this project is to ascertain whether dysbiosis of the gut microbiome contributes to the development of progressive MS. To test this idea, we will use the 1C6 T cell receptor transgenic mice that initially displays an acute EAE (experimental allergic encephalomyelitis) phenotype that subsequently becomes progressive.

Thus far, we have observed that 1C6 animals display two EAE phenotypes in both females and males - progressive and non-progressive – and that male progressive animals display weaker grip strength. To determine if modulation of the gut microbiota affects the development of progressive or non-progressive disease, 1C6 EAE animals were administered a cocktail of antibiotics after the first acute phase. Antibiotic treatment markedly increased not only disease severity but the ratio of animals that developed progressive (100%). We are currently performing 16S sequencing to determine the microbota composition of animals with and without antibiotic treatment. If any bacterial species correlate with progressive EAE, their role in promoting progressive disease will be tested in germ-free 1C6 mice and via fecal microbiota transplantation.

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#45 Disease-Modifying Therapies Used to Treat Multiple Sclerosis and the Gut Microbiome: A Systematic Review

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Background: The gut microbiome may play an important role in multiple sclerosis (MS). However, its relationship with the disease-modifying therapies (DMTs) use remains unclear. We systematically reviewed the literature to examine the relationship between DMTs and gut microbiota composition and functionality among persons with MS (pwMS).

Methods: MEDLINE, EMBASE, Web of Science, and Scopus were searched (01/2007-09/2022) for studies evaluating potential differences in diversity or taxonomic relative abundances of the gut microbiota between DMT

exposed/unexposed pwMS or before/after DMT initiation. All DMTs approved by the US Food and Drug Administration for MS (1993-09/2022) and rituximab were included. We summarized differences in gut microbiota diversity, relative abundances, and functional capacity between DMT exposed and unexposed pwMS.

Results: Of 410 studies, 11 were included; 9 were cross-sectional and 2 longitudinal, totalling 1243 pwMS. Of these, 821 were exposed to a DMT and 473 were unexposed, including 51 assessed before and after DMT initiation. DMT use duration ranged from 14 days to >6 months. All studies examined stool; 9 used 16S rRNA sequencing, 1 metagenomics, and 1 both. Risk of bias was low for 9 and medium for 2 studies. Gut microbiota alpha-diversity was compared between DMT users/non-users in 8 studies, and none found a significant difference ($p>0.05$). Beta-diversity was examined in 5 studies; 1 observed a difference in interferon-beta users vs. DMT non-users only (weighted UniFrac, $p=0.006$). All studies examined taxa-level differences, but most (6) combined different DMTs. Two or more studies reported 7 genera (Bacteroides, Clostridium sensu stricto 1, Haemophilus, Megasphaera, Pseudomonas, Ruminiclostridium 5, Turicibacter) and 1 species (Ruthenibacterium lactatiformans) differing in the same direction between DMT users/non-users. Three studies assessed functionality, reporting lower relative abundances of carbohydrate degradation and reductive tricarboxylic acid cycle I pathway in DMT users vs. non-users ($p<0.05$), but findings could not be attributed to a specific DMT.

Conclusion: While DMT use (versus no use) was not associated with differences in gut microbiota diversity across most studies, taxa-level differences were observed. However, most studies were cross-sectional, few examined functionality, and mechanistically different DMTs were often combined. Further work is warranted as it may help identify potential therapeutic targets to optimize MS management.

Keyword: *multiple sclerosis, gut microbiota, disease-modifying therapy, systematic review*

#69 Dietary methionine restriction limits neuroinflammatory and neurodegenerative processes in preclinical models of multiple sclerosis

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Background: Multiple Sclerosis (MS) is a chronic inflammatory and demyelinating disorder of the central nervous system (CNS) more common in women than men. Dietary methionine restriction (MR) displays anti-inflammatory properties and improves metabolic health through sexually dimorphic mechanisms. We demonstrated that methionine pathway is induced upon T cell activation in vitro and that MR affects the effector function and proliferation of TH17 lymphocytes, considered pathogenic in MS and its animal model, experimental autoimmune encephalomyelitis (EAE).

Objective: To study the manipulation of T cell methionine metabolism as a new therapeutic avenue for controlling neuroinflammatory diseases such as MS in both sexes.

Methods: We immunized C57BL/6 mice exposed to low methionine (MR) vs. control diet with MOG to induce active EAE and used transgenic TCR1640 mice developing spontaneous EAE and

exposed to MR vs. control or methionine supplemented (M+) diet to test the impact of methionine intake on clinical course and immune cell distribution and activation (flow cytometry and RNAseq). We assessed the impact of methionine intake on the composition of the gut microbiota with 16S rRNA-sequencing. Finally, serum neurofilament light chain (sNfL) levels were measured to evaluate neuroaxonal injury.

Results: MR delays onset of neurological deficits in active EAE. This is paralleled by lower numbers of peripheral and infiltrating immune cells and pro-inflammatory lymphocytes such as CD4 cells expressing CD49d, CD44, CD146 and CD196 at the presymptomatic (day 7 post-induction), pre-onset (day 10) and peak (day 15) stages. Moreover, MR delays onset of spontaneous EAE in TCR1640 mice, with a near complete abrogation in males. This is associated with lower numbers of immune cells and pro-inflammatory lymphocytes as well in the spleen and CNS during the presymptomatic, early symptomatic and chronic phases. In addition, the elevation of sNfL observed at peak of active EAE is reduced in mice exposed to MR, with a more pronounced impact on females. Similarly, we found reduced levels of sNfL as well in mice exposed to MR during the chronic phase of spontaneous EAE, while M+ diet is associated with increased levels in females. Finally, the composition of the gut microbiota is influenced by diet and biological sex and could mediate the beneficial impact of MR on neuroinflammatory processes.

Conclusion: MR ameliorates EAE clinical course and limits neuroinflammatory processes and neuroaxonal injury in two MS preclinical models.

#134 Lifestyle factors modulate disease severity in a murine model of multiple sclerosis via hematopoiesis

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Multiple sclerosis (MS) is an autoimmune disease driven by immune cell infiltration of the central nervous system. Immune cells are produced in the bone marrow in a process called hematopoiesis. In this study, we aimed to evaluate the contribution of hematopoiesis to the progression of MS. Using the experimental autoimmune encephalitis (EAE) model of MS, we report that hematopoiesis is increased in the vertebrae and femur bone marrow of affected animals compared to naive baseline. Interestingly, extramedullary hematopoiesis in the spleen was also increased in the EAE model. Exacerbation of hematopoiesis by Apoe deletion and high fat diet feeding increased immune cell generation and infiltration of the spinal cord, and worsened EAE clinical score. By contrast, suppression of hematopoiesis with voluntary exercise improved clinical scores in EAE mice. This cannot be attributed to peripheral priming as analysis of $CD4^+$ T cells in the spleen and cervical lymph nodes during the onset phase showed no differences in growth factor production or proliferation. Instead, we report increased production of growth factors in the femur bone marrow. Collectively, these data indicate that hematopoiesis promotes MS progression and modulates disease severity. We propose that normalization of hematopoiesis in MS patients through promotion of a healthy lifestyle, such as

low-fat diet and regular exercise, may alleviate MS symptoms.

Keyword: *multiple sclerosis, lifestyle factors, hematopoiesis*

#170 SILYMARIN INCREASES CORTICAL BDNF CONTENT IMPROVING POST-CEREBRAL ISCHEMIA SURVIVAL IN OBESE MICE

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Stroke is characterized by acute focal brain injury caused by vascular factors, leading to neurological deficits.Several pathological changes accompany cerebral ischemia, including neuroinflammation and neurotrophic factors dysregulation. Epidemiological studies indicate that around 90% of ischemic strokes can be attributed to modifiable risk factors like obesity. Obesity elevates the risk of metabolic and cardiovascular diseases and contributes to persistent systemic low-grade inflammation, which can subsequently lead to secondary neuroinflammation, exacerbating the consequences of stroke.

Silymarin, obtained from the plant Silybum marianum, possesses anti-inflammatory, antioxidant, and neuroprotective properties and thus could reduce neuroinflammation secondary to obesity or stroke-derived damage. This work aimed to asses whether obesity-induced neuroinflammation can be a factor in cerebral ischemia prognosis and if oral administration of silymarin can modulate it.

Male C57/Bl6 mice were fed a high-fat diet (HFD) or standard chow diet (ND) for 12 weeks. Fasting glucose, intraperitoneal glucose, and insulin sensitivity tests were determined after 12 weeks of diet. 100 mg/kg of silymarin was administered orally daily for 14 days. HFD-induced obese mice were submitted to photothrombosis to induce cerebral ischemia in the motor cortex and then treated for 14 days with silymarin. Cortical and striatal BDNF, MCP1, TNFα, CX3CL1, and IL10 levels were determined with ELISA.
Our study demonstrated that silymarin treatment effectively restored glucose and insulin sensitivity in obese mice. Furthermore, this restoration was accompanied by a reduction in pro-inflammatory signals such as TNFα and MCP1 and modulation of the BDNF content. HFD mice presented higher mortality after photothrombosis-induced cerebral ischemia than control mice and silymarin treatment diminished mortality in both groups. Interestingly, the ND and HFD groups exhibited increased levels of pro-inflammatory signals like MCP1 and CX3CL1 within the ischemic groups. However, silymarin treatment restored CX3CL1 levels, increased the BDNF content in the cortex, and reestablished it in the striatum.
These findings suggest that silymarin may act as a neuroprotective agent by modulating the content of BDNF and cytokines, as well as regulating glucose metabolism and insulin sensitivity.

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Keyword: *Cerebral ischemia, obesity, silymarin, BDNF, CX3CL1*

#209 Identification of Pathogenic Bacteria Underlying Multiple Sclerosis Progression

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Background: Multiple sclerosis (MS) is an autoimmune demyelinating disease. Some relapsing-remitting MS (RRMS) patients shift to secondary progressive MS (SPMS) which is characterized by accumulating disabilities and resistance to medications. We and others previously revealed the distinct features of gut microbiomes between RRMS and SPMS (Takewaki et al. PNAS. 2020). However, it remains unclear whether gut dysbiosis causes MS progression.

Objectives: To identify the pathogenic gut bacteria associated with MS progression and reveal the specific mechanism which exacerbates neuronal inflammation.

Methods and Results: We compared the fecal microbiome between 62 RRMS patients and 15 SPMS patients based on the short-read metagenomic data. We identified bacteria X whose abundance was significantly enriched in the SPMS group and most positively correlated with a neurological disability score of patients. The significant increase of bacteria X in the progressive MS group was confirmed by publicly available large-scale metagenomic data provided by international joint research in western countries (iMSMS Consortium. Cell. 2022). At the strain-level analysis based on long-read metagenomics, we newly identified a distinct cluster of bacteria X strains which was highly enriched in the SPMS group. Mono-colonization of this novel bacteria X strain into germ-free experimental autoimmune encephalomyelitis mice caused exaggerated neurological disability (p=0.0007) with significantly increased T helper 17 (Th17) cells in both colon and central nervous system compared with controls (**Figure 1**). Among various bacteria X strains, the genome of this novel strain specifically included flagellar genes, which was confirmed by a scanning electron microscopic imaging analysis. Finally, several immunological experiments suggested that this novel strain-derived flagella induce pathogenic Th17 cells in the gut possibly via the combination of toll-like receptor 5 stimulation

and promotion of bacterial adherence to colonic epithelial cells (**Figure 2**).

Conclusions: Bacteria X enriched in the gut of SPMS patients exacerbates neuronal inflammation via flagella-Th17 axis. The selective elimination of pathogenic bacteria including bacteria X might lead to the innovative therapy for SPMS patients.

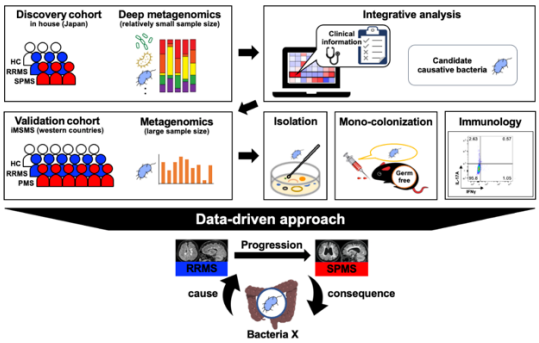

Figure 1. Conceptual diagram of this research.

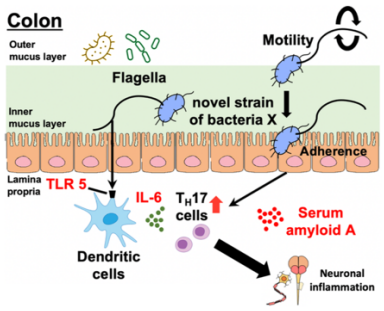

Figure 2. Schematic representation of specific mechanism that bacteria X (novel strain) exacerbates neuronal inflammation.

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Keyword: *Progressive multiple sclerosis, Microbiome, Long-read metagenome, Gnotobiote, Flagella*

#338 STUDY OF THE EFFECTS OF TREHALOSE ON THE ENTERIC AND CENTRAL NERVOUS SYSTEM IN A TRANSGENIC MOUSE MODEL OF PARKNISON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder affecting the dopaminergic neurons in the nigrostriatal region of the brain and causes neuronal inclusions mainly composed of the protein alpha-synuclein (αSYN). The available treatments are only symptomatic. The disease manifests as motor symptoms, which are often preceded by non-motor symptoms such as gut microbiota disturbances and constipation. These could be related to damage to the enteric nervous system. According to Braak's hypothesis, PD could develop in the intestine and then spread to the brain through the vagus nerve. Thus, peripheral neurodegeneration and inflammation could be the key to better understand the origin and progression of PD down to the level of the central nervous system.

Trehalose is a sugar found in fungi that has shown neuroprotective effects in various models of neurodegenerative diseases. However, its mechanism of action is still unclear. The transport mechanism of trehalose is still poorly understood. As it does not possess a membrane transporter and is not synthesized endogenously in vertebrates, the neuroprotective effect of

trehalose would be mediated by a change in the intestinal microbiota. Therefore, we aim to validate the neuroprotective potential of trehalose through its intestinal action.

We used transgenic mice that overexpress human αSYN as a model of PD. We treated them with drinking water or water containing trehalose, maltose or sucrose (2%, w/vol) for seven months. We performed behavioral tests and collected feces to analyse fatty acids and microbiota. We also carried out postmortem histopathological analyses of the brain and intestine.
The preliminary results show that transgenic mice exhibit hyperactivity, stress, anxiety, constipation, which are also found in Parkinsonian patients. They also show a decrease in striatal tyrosine hydroxylase (an enzyme involved in dopamine synthesis), which is prevented by trehalose treatment. The gut microbiota analysis reveals a significant difference in microbial diversity between the two genotypes, and that trehalose affects the composition of the microbiota, mainly enrichment of bacteria of the genus lachnospiraceae.

In conclusion, trehalose has promising neuroprotective effects that could be mediated by the microbiota. This work has a significant impact for PD patients, as it could lead to a nutraceutical clinical study on trehalose in the future.

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Keyword: *Parkinson Disease, Dopaminergic, Trehalose, Microbiota, Alpha-synuclein*

#356 Understanding the role of the gut microbiota and intestinal inflammation in Parkinson's disease

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Parkinson's Disease (PD) is a common chronic neurodegenerative disorder characterized by loss of motor function resulting from the death of dopaminergic neurons in the substantia nigra region of the brain. The exact etiology of PD is unknown, however, there is increasing evidence that shows that PD is an autoimmune disorder. One gene involved in PD is PTEN-induced kinase 1 (PINK1), which has been recently found to repress mitochondrial antigen presentation (MitAP) on major histocompatibility complex class I molecules. Our lab and collaborators have previously shown that, in the context of an infection with Citrobacter rodentium, which imitates enteropathogenic Escherichia coli infection, $Pink1^{-/-}$ mice had significantly greater MitAP, leading to a greater induction of autoreactive CD8+ T cells. Additionally, these mice exhibited a motor deficit in a pole test 6

months post C. rodentium infection. Preliminary studies from our lab suggest that the gut microbiome is important in PD phenotype. We have observed that non-rederived "dirty" mice, which are positive for mouse commensal Helicobacter species, experience a strong induction of autoimmunity, whereas rederived "clean" mice, which are no longer Helicobacter positive, do not. Helicobacter is a Gram-negative genus which infects the gastric and intestinal mucosa and is associated with chronic inflammation. Many clinical studies have found a link between Helicobacter pylori infection and PD. The infection and eradication of H. pylori have also been associated with changes in intestinal and fecal microbiota diversity. Based on the literature and previous findings from our lab, we hypothesize that the induction of PD is a three-hit model composed of genetic susceptibility, microbiome permissiveness, and intestinal inflammation. We have already seen that the baseline microbiome is not different between wild-type and Pink1$^{-/-}$ mice, though we do see some sex differences, notably in the phyla Verrucomicrobiota and Desulfobacterota, which are more abundant in male mice. Additionally, we have seen a greater richness in the "dirty" mice, and greater diversity across the Gram-negative phyla of the dirty mice. We next seek to determine whether colonization with the commensal Helicobacter hepaticus is sufficient and necessary to induce MitAP in "clean" mice after they undergo intestinal inflammation by C. rodentium infection. We also seek to determine how H. hepaticus colonization affects the gut microbiome.

Neuroimmunology general II

#214 Distinct features of B cell receptors of neuromyelitis optica spectrum disorder among central nervous system inflammatory demyelinating diseases: Bulk BCR analysis in PBMC

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Background

B cells play an important role in the development, progression, and relapse of antibody-mediated autoimmune diseases. Each B cell expresses a single B cell receptor (BCR), a membrane bound form of antibody, and analysis of BCR repertoire can provide insights into the B cell-associated pathophysiology of the relevant diseases. In the central nervous system (CNS) inflammatory demyelinating diseases (CIDDs), characteristics of BCR may differ by the presence and type of autoantibodies. In this study, using high-throughput sequencing, we investigated BCR features of patients with CIDDs in relation to their antibody status.

Methods

We collected blood samples for BCR analysis from the prospective cohort of CIDDs in a tertiary referral center. We grouped the patients according to results from cell-based assays for

auto-antibodies: patients with anti-AQP4 antibody (NMOSD), those with anti-MOG antibody (MOGAD), and those with double-seronegative demyelinating diseases (DSN). We analyzed the heavy chain (IGH) of BCR in peripheral blood mononuclear cell (PBMC). PBMCs were isolated from blood samples and total RNA was extracted from each sample. cDNA libraries were synthesized with primers specific to the constant region and sequenced using NovaSeq. We analyzed and compared BCR features including isotype class, clonality, somatic hypermutation (SHM), and complementarity-determining region 3 (CDR3) length between the disease groups.

Results

BCR IGH repertoire analysis was performed on a total of 33 patients with CIDDs (13 NMOSD, 12 MOGAD, and 8 DSN) and 34 healthy controls. All the CIDDs groups showed more activated BCR features than healthy controls, particularly more pronounced in the NMOSD group. Compared to the other CIDDs groups, NMOSD patients had a lower proportion of unswitched isotypes, while a higher proportion of IgG1. Additionally, NMOSD patients showed the most expanded clonality and the highest SHM rate, suggesting the greatest B cell activation. Within the NMOSD patients, higher clonality and SHM rate were associated with older age and longer disease duration.

Conclusion

NMOSD revealed the highest level of B cell activation, as indicated by increased isotype class switching, clonality, and SHM rate among CIDDs, suggesting that BCR features of NMOSD are distinct from those of other CIDDs including MOGAD.

#236 Effect of carnosic acid-loaded theranostic nanocarriers on the oxygen-glucose deprivation-induced cell damage and inflammatory profile in organotypic hippocampal cultures.

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The complex pathophysiological cascade of ischemic neuronal death comprises a variety of distinct pathological events, including oxidative stress and neuroinflammatory processes, which may be targeted by pharmacotherapeutic strategies. However, an inefficient delivery of drugs to the stroke-affected part of the brain due to the blood-brain barrier and peripheral adverse effects limit their potential clinical usefulness. Therefore, the targeted and monitored delivery of neuroprotectants with the right dosage is considered a key issue in the future treatment of ischemic stroke. To address this problem, we designed theranostic nanocarriers that can cross the blood-brain barrier without imposing side effects on its normal function. In the present study, we tested carnosic acid (CA) as promising neuroprotective and anti-inflammatory compound encapsulated into AOT (liquid core) and PCL (solid core) theranostic nanocarriers in the oxygen-glucose deprivation (OGD) procedure in the organotypic hippocampal cultures (OHCs), which is commonly accepted ex vivo model for ischemic stroke. The biocompatibility of two types theranostic nanocarriers without/with Gadolinium (Gd), empty and CA loaded was tested.

OHCs were prepared from the hippocampi of 6-7-day-old Wistar rat pups. On the 7th day in vitro, slices were pre-treated with various theranostic nanocarriers. For the OGD procedure, OHCs were incubated in preequilibrated and deoxygenated medium in a hypoxic chamber. After OGD, the slices were transferred to a fresh oxygenated culture medium and placed in normoxic conditions. The effect of nanocarriers on time-dependent OGD-induced changes in the lactate dehydrogenase (LDH) release, nitric oxide (NO) synthesis, the level of inducible factor HIF-1alpha and pro- and anti-inflammatory factors were evaluated. Results showed that exposure of hippocampal slices to OGD led to significant up-regulation of LDH and NO release. It also increased HIF-1 alpha level and proinflammatory factors (IL-1 beta, IL-18, IL-6, TNF-alpha, CCL3, CXCL10 and CCL5) release. Simultaneously OGD decreased anti-inflammatory (IL-4, IL-10) cytokine production. Importantly both types of the tested empty nanocarriers neither showed cytotoxicity nor ability to modulate immune mediator profile in control and OGD-treated OHCs. We also found that the AOT/Gd loaded with CA diminished the HIF-1 alpha release and some pro-inflammatory cytokines in OGD-OHCs model. Overall, results of the present study suggest that the designed theranostic nanocarriers are biocompatible in terms of their apparent lack of neurotoxicity and immunotoxicity. Moreover, they might be useful as nanocarriers of some neuroprotective and anti-inflammatory drugs to the ischemia-affected brain region. However, the quite promising properties of the theranostic nanocarriers need to be confirmed in an in vivo study.

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Keyword: *theranostic nanocarriers, carnosic acid, oxygen-glucose deprivation*

#237 Unexpected remyelination in the absence of matrix metalloproteinase 7

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Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system, characterized by focal lesions with varying degrees of inflammation, demyelination, glial scar formation and axonal loss. As MS progresses, remyelination fails, leaving neuronal axons vulnerable to degeneration and resulting in permanent neurological disability. In chronic MS lesions, the aberrant accumulation of extracellular matrix (ECM) molecules, including fibronectin and hyaluronan, impairs oligodendrocyte progenitor cell (OPC) differentiation, contributing to remyelination failure. Removing this inhibitory ECM is a therapeutic target to stimulate remyelination. Intriguingly, expression of the fibronectin-degrading enzyme matrix metalloproteinase 7 (MMP7) is decreased in chronic MS lesions compared to control white matter. Therefore, we examined the response to cuprizone-induced demyelination in MMP7$^{-/-}$ mice, hypothesizing that lack of MMP7 would lead to impaired breakdown of ECM, including fibronectin, and hence remyelination failure. Cuprizone

intoxication led to inflammation and overt loss of oligodendrocytes in both MMP7$^{-/+}$ and MMP7$^{-/-}$ mice. However, contrary to expectation, remyelination proceeded efficiently in the absence of MMP7. In the remyelination phase, lack of MMP7 did not lead to accumulation of fibronectin or laminin, another MMP7 substrate. Intriguingly, in the setting of chronic demyelination, levels of fibronectin as well as hyaluronan, which is not known to be degraded by MMP7, were actually lower in MMP7$^{-/-}$ mice. Overall, our results indicate that MMP7 is not critical for remyelination in the cuprizone model and point to a moonlighting function for MMP7 in fibronectin and hyaluronan production during chronic demyelination. These findings highlight the complexity of the MMP system and suggest that absence of MMP7 is not the sole cause of fibronectin accumulation in MS lesions.

Keyword: *Matrix metalloproteinase 7, extracellular matrix, fibronectin, multiple sclerosis, remyelination*

#242 Establishment of Decellularized Region-Specific Brain Scaffolds for Investigating the Effect of ECM on Myeloid Heterogeneity and Endogenous Oligodendrogenesis

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Neuroinflammation and CNS insults may lead to alterations of the extracellular matrix (ECM) that in turn can influence regenerative processes such as oligodendrocyte recovery and function. Specific ECM matrisome proteins were identified to hinder or promote processes required for remyelination. In line with this, this project aims to establish a decellularization method to generate human brain ECM scaffolds and to investigate the effect of brain ECM on monocyte phenotype and neural stem cell (NSC) differentiation.

We have successfully established a decellularization method on post mortem human brain regions containing stem cell and non-stem cell niches. This working model, which displays complete removal of nuclear DNA and RNA while still preserving matrisome proteins, was used as a biological scaffold for recellularization and cell-based assays.

Proper integration of monocytes and NSCs was observed after reseeding onto the region-specific ECM scaffolds. The FlowSOM analysis reveals monocytic clusters with inter-individual phenotype variation depending on the human brain region that was used as scaffold. However, the most frequent cell clusters in each region displayed an anti-inflammatory phenotype. On the other side, after successful attachment and differentiation, the NSCs appeared to express neuronal, astrocytic, and markers leading to the oligodendrocyte lineage depending on the brain region.

Thus, this ECM model will allow to test and compare how myeloid cell phenotype changes and how CNS progenitors differentiate on different brain regions. This will be instrumental to understand the link between biochemical properties of diseased brain tissue with functional myelination and CNS regeneration in an ex vivo setting.

Keyword: *ECM, Decellularization, CNS Regeneration, Monocytes, Neural Stem Cells*

#250 Multi-omics profiling reveals novel pathways influenced by the cerebellar degeneration-related proteins in ovarian cancer

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Paraneoplastic neurological syndromes are immune-mediated disorders triggered by cancer, characterized by circulating antibodies directed against antigens expressed by neurons and tumour cells. Of these rare disorders, paraneoplastic cerebellar degeneration (PCD) is one of the most common forms. In patients with PCD, the dominant paraneoplastic antibody is anti-Yo, detected in serum and cerebrospinal fluid (Shams'ili, 2003). Anti-Yo targets three intracellular antigens expressed by Purkinje neurons - cerebellar degeneration-related protein 1 (CDR1), CDR2 and CDR2-like (CDR2L). The interaction between anti-Yo and CDR proteins is thought to mediate Purkinje neuron dysfunction and death, causing a subacute onset of limbic and truncal ataxia, dysarthria and nystagmus (Darnell, 2003). Expression of the CDR proteins are also seen in the associated tumours, most often ovarian epithelial cancer and breast cancer (McKeon, 2011). Tumor expression of the CDR proteins is believed to trigger an autoimmune response targeting both cancer cells and Purkinje neurons.

Our understanding of the biological functions of the CDR proteins are limited. Consequently, the mechanisms by which the CDR proteins contribute to the pathogenesis of PCD remain unknown. To gain a deeper understanding of the biological functions of these proteins, we generated CDR1, CDR2 and CDR2L knockout ovarian cancer cell lines, and applied a multi-omics approach to analyse changes in the transcriptome and proteome. Our analysis revealed several novel pathways influenced by the deletion of the CDR genes. In CDR1 and CDR2L knockout cells, we observed differential expression across all omics layers of several key genes involved in immune-related processes. Our findings help shed light on the biological functions of these cryptic proteins.

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Keyword: *PCD, PNS, Cerebellar degeneration, CDR2L, CDR2*

#258 A proteomic study to unveil IL-1β-dependent neurotoxicity in experimental autoimmune encephalomyelitis

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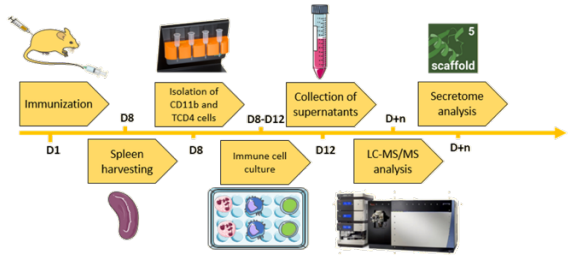
Background: The exact causes of multiple sclerosis (MS) are still poorly understood. Several factors, such as immune system dysfunction, genetics and the environment, seem to be involved in the disease. The presence of the proinflammatory cytokine interleukin (IL)-1 beta was previously correlated with the number and volume of brain MS lesions. Our recent work has demonstrated that the interaction between IL-1beta-producing monocytes and $CD4^+$ T lymphocytes results in the production of factors that are toxic to neurons.

Objective: The aim of this project is to identify the proteins responsible for the neurotoxic effects of IL-1 beta throughout the development and progression of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS.

Methods: To achieve this goal, novel quantitative proteomics approaches will be used to identify proteins mediating neuronal toxicity from conditioned media derived from myelin-reactive CD4+ T cells (2D2) cultured in the presence of IL-1 beta-competent myeloid cells.



Results: We observed that IL-1 beta derived from myeloid cells participates in CD4+ T cell activation. We also demonstrated that conditioned medium derived from IL-1 beta-competent myeloid cells co-cultured with myelin-reactive CD4+ T cells significantly inhibits neurite outgrowth of embryonic cortical neurons.

Conclusion: This project could help identify some of the IL-1 beta-dependent protagonists of EAE, and therefore potentially new therapeutic targets. We also aim to use the techniques that we will develop to validate our findings in human cells, since they could provide a simple, non-invasive method for assessing the contribution of immune cells to CNS damage in MS patients.

Keyword: *Neurotoxicity, Proteomics, IL-1 beta*

#269 Interleukin 4 signaling is required for myelin compaction around axons

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Interleukin-4 (IL-4) has been shown to have beneficial effects on neuroinflammatory axon injury (1). IL-4 receptor (IL-4R) has been found to be expressed in microglia, neurons, oligodendrocytes, and Schwann cells (2). However, the role of IL4R signaling in axon myelination is not clear. In this work, we show that disrupting IL-4 signaling by knocking down the IL-4R using morpholinos in zebrafish leads to defects in the spinal cord, and an altered mRNA signature consistent with aberrant myelination and activation of pro-inflammatory pathways. Morphants in which IL-4R was knocked developed spontaneous lesions in the spinal cord, associated with macrophage infiltration. Further, we observed that IL-4R knockdown caused axon hypertrophy and decreased myelin compaction. Quantitative polymerase chain reaction (PCR) and in-situ hybridization revealed increased myelin basic protein (mbp) and oligodendrocyte transcription factor 2 (olig2). In addition, there was an increase in proinflammatory interleukin 1-beta (IL-1b) and nitric oxide synthase 2b (nos2b). In contrast, the anti-inflammatory interleukin 10 (IL-10) was decreased. Our results suggest an essential role of IL-4 signaling in myelin compaction around axons in the central nervous system.

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Keyword: *Interleukin, Central nervous system, Myelin, Zebrafish*

#272 Context-dependent transcriptional regulation of microglial proliferation

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Microglia, are the resident macrophages of the central nervous system (CNS), have several important functions which are essential for the normal development, maintenance, and homeostasis of the CNS. Disruption of microglia function is associated with multiple neurodegenerative and neurodevelopmental diseases. Both in a neurodevelopmental and neuropathological context, microglia proliferate massively. In the first case, their primary goal is to colonize the CNS and participate in its development. In the second case, microglia proliferate to protect the CNS and to maintain its homeostasis. Microglia proliferation requires finely orchestrated transcriptional regulation in order to appropriately coordinate the expression of several proliferation-related genes. We investigated and compared the transcriptional mechanisms associated with microglia proliferation in postnatal development and in an adult model of microglia depletion-repopulation. The data show that proliferating microglia induce the transcription of genes associated with the proliferation environment, as well as two groups of genes associated with proliferation. The first group of genes is already expressed in quiescent microglia and is regulated by the transcription factors Klf/Sp, Nfy and Ets. The second group represents genes that are newly induced upon entry into proliferation, and whose regulation involves the transcription factors Lin54 and E2f. Altogether we show that the transcriptional

program associated with microglia proliferation is "context-dependent."

Keyword: *Microglia, Proliferation, Transcriptional regulation*

#279 Central Delivery of Interleukin-1α Induces Blood-Spinal Cord Permeabilization and Immunoglobulin G Infiltration in the Mouse Spinal Cord and Brain

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Spinal cord injury (**SCI**) can be divided into primary damage caused by mechanical tissue disruption and secondary damage initiated by the release of alarmins from necrotic cells into the spinal cord parenchyma, triggering neuroinflammation. During the secondary damage phase, the blood-spinal cord barrier (**BSCB**) undergoes changes that increase its permeability. Our recent work revealed that the alarmin IL-1alpha is released by necrotic microglia almost immediately after SCI and has a deleterious role in inflammation and the pathology. To recapitulate these processes in vivo, we injected recombinant mouse IL-1alpha intra-cisterna magna (**i.c.m.**) into adult mice to study its effects on the spinal cord and brain. We found that IL-1α induces infiltration of neutrophils, disruption of the BSCB, and the death of mature oligodendrocytes within 24 hours after injection. Notably, the permeabilization of the BSCB was correlated with the infiltration of immunoglobulin type G (**IgG**) and their internalization inside neurons of the spinal cord, midbrain, and brainstem. To demystify the mechanisms by which IgG enters into neurons in these regions, we performed injections of IL-1alpha in a murine model devoid of all activating Fc gamma receptors (**FcγR$^{-/-}$**). Surprisingly, our investigation revealed a similar occurrence of IgG infiltration into CNS neurons in this model, indicating that activating FcγRs do not play a significant role in this sequestration process. To further investigate the relationship between neurons and IgG, we conducted i.c.m. injections of polyclonal IgG conjugated to Alexa 488 in adult control mice, allowing us to assess if this IgG internalization may occur in non-pathological conditions. Our findings revealed a notable sequestration of IgG within neurons in the frontal region of the brain, the cortical amygdalar region, and throughout the spinal cord at both the 1-hour and 4-hour time points post-injection. We replicated the injections using Alexa 488-conjugated IgG in FcγR$^{-/-}$ mice, and once again observed IgG infiltration in the aforementioned regions. This strongly suggests that neurons possess the capacity to sequester IgG, even in normal physiological conditions, and that this process is not mediated via activating FcγRs. The implications of this neuronal capability may be far-reaching, particularly in pathologies characterized by elevated circulation of IgG within the CNS and cerebrospinal fluid, such as in SCI and systemic lupus erythematosus.

#304 An investigation of strain, sex, and reagents for EAE protocol establishment

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Experimental autoimmune encephalomyelitis (EAE) is the most common animal model of multiple sclerosis (MS). Several factors such as strain, age, microbiota, circadian rhythm, and sex

of the animals, as well as seasonality and protocols of immunization have been reported to influence the induced disease course. This can provide great flexibility and the possibility to induce a specific disease course (use of SJL mice for relapsing-remitting course for instance). However, it also leads to increased variability and difficulty in establishing robust protocols within the field, and often makes it challenging to compare treatment outcomes across labs, and to translate findings to other species.

In this context, we have qualified a protocol for EAE in C57BL/6 mice immunized with MOG_{35-55}, and examined the effect of strain, sex, and different sets of reagents on several disease features to find the most robust combination.

We found that we could induce EAE in C57BL/6J mice with higher incidence than in C57BL/6N mice. In addition, comparison of two C57BL/6J substrains from Taconic (C57BL/6J BomTac) and Charles River (C57BL/6J Crl) revealed that although both substrains reached similar disease severity overall, the C57BL/6J BomTac mice developed a more robust form of disease, with less variable onset, maximum grade, and disease length. Surprisingly, induction of disease in male mice led to higher incidence, with no significant difference at onset or in disease severity. Finally, we show that emulsions and Bordetella pertussis toxin origin makes a difference in nearly all disease parameters.

While these findings have helped us establish a robust model of EAE in-house, it is important to consider that the health status of different animal facilities and the associated differences in gut microbiota, as well as the different animal providers available locally can be a significant barrier to translating results obtained from one laboratory to another. Overall, our conclusions aim at encouraging EAE users to qualify their model thoroughly to achieve the best robustness in their setting.

Keyword: *Experimental Autoimmune Encephalomyelitis, model, strain, sex*

#315 Smoking status in relation to fluid biomarkers in acute optic neuritis

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Introduction:
Smoking has been linked to accelerated disease progression in multiple sclerosis (MS), but the involved mechanisms remain unclear. As smoking cessation is associated with normalised rates of progression, we hypothesise that a better understanding of the effects of smoking may reveal modifiable drivers of MS progression.

Objective:
The aim of this study is to elucidate whether and how smoking affects fluid biomarkers in patients with acute optic neuritis.

Methods:
Questionnaires were used to classify patients with acute optic neuritis (ON) that had a lumbar puncture performed in the years 2018-2021 as daily smokers, occasional smokers, former smokers or never smokers. Furthermore, data on cerebrospinal fluid (CSF) white blood cell counts (WBCs), IgG-index, Qalb (albumin quotient between CSF and serum x 10^{-3}) and serum 25-hydroxy-vitamin-D were collected. Results are presented as medians with interquartile range.

Wilcoxon rank sum test and multiple regression were used to study the effects of smoking.

Results:
We recruited 75 patients with acute ON (61% MS-converters, 65% women, age: 32 (27-40) years) with the following distribution among the smoking status groups: 19 daily smokers, 5 occasional smokers, 15 former smokers and 36 never smokers. We found no differences in CSF WBCs and IgG-index among groups. Daily smokers had higher Qalb (5.6 (4.3-6.8)) compared to never smokers (4.1 (3.2-4.8), p=0.005) and former smokers (3.7 (3.0-5.3), p=0.03). We found no difference in Qalb between never smokers and former smokers (p=0.96). Daily smokers had much lower serum IgG (9.4 (8.4-10.2 g/L) compared to never smokers (11.7 (10.4-12.9) g/L, p=0.00002), but not significantly lower than former smokers (10.0 (8.8-11.7) g/L, p=0.09). The difference in serum IgG between former smokers and never smokers did not reach significance (p=0.06). Daily smokers had lower serum vitamin D (37 (25-49) nmol/L) compared to never smokers (48 (32-70) nmol/L, p=0.029) and former smokers (62 (36-78) nmol/L, p=0.024). We found no difference in serum vitamin D between former smokers and never smokers (p=0.41). The effects of smoking on Qalb, serum IgG and serum vitamin D were still significant after adjusting for MS-diagnosis, age and gender.

Conclusion:
The above data suggest that smoking is associated with increased permeability between the blood and the CSF, reduced systemic humoral IgG immunity and lower levels of serum vitamin D in acute ON. Future studies should investigate the relationship between the suggested mechanisms and disease progression in MS.

Keyword: *optic neuritis, smoking*

#316 Human pluripotent stem cell-based models dissect the cellular neurotropism and neurovirulence of Monkeypox virus

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Monkeypox virus (MPXV) belongs to the Orthopoxviridae family and is included in the same genus as the variola virus, vaccinia virus, and cowpox virus. MPXV is the leading cause of Mpox, a zoonotic disease that was first detected in humans in 1970 (Parker & Buller, Future Virol, 2013), when it caused sporadic outbreaks in western and central Africa. It became epidemiologically relevant in July 2022 and declared a Public Health Emergency by WHO with over 86000 cases worldwide. MPXV infects humans via respiratory and dermal route causing flu-like symptoms that include fever, myalgia, and a characteristic rash (Mitjà et al, The Lancet, 2023). Complications of human MPXV infection include sepsis, pneumonia, and neurological sequelae. In approximately 2-3% of cases, and in the majority of severe cases, patients present with neuropsychiatric complications including

seizures, confusion, and encephalitis suggesting MPXV can invade the central nervous system (CNS) (Billioux et al, JAMA Neurol, 2022; Cole et al, Lancet Infect Dis, 2022). Currently, the neuropathogenesis of MPXV is largely unknown, and there are no available treatments for Mpox-associated encephalitis. In this study, we investigate MPXV neurotropism by using human pluripotent stem cell (hPSC)-derived cortical neurons, astrocytes and microglia. We show that while all cell types are susceptible to viral infection, virus-induced phenotypes are cell type-specific. Cortical neurons in vitro were the least permissive cell type, meaning that despite initial infection, cortical neurons restricted viral replication. Astrocytes and microglia were susceptible and permissive, meaning that viral particles were able to replicate over time. Intracellular viral particles were observed in these cells by electron microscopy. Unlike other cell types, MPXV-infected astrocytes showed severe cytopathogenic effects and immune activation. Transcriptional profiling of mock- and MPXV-infected astrocytes highlighted the dysregulation of genes associated to senescence and senescence-associated secretory phenotype (SASP), suggesting that viral infection could induce an arrest of the cell cycle, and direct astrocytes towards a neuroinflammatory phenotype. Viral infection of astrocytes, neurons and microglia was detected after the incubation of MPXV with primary human brain tissue, which confirmed what was seen in the in vitro hPSC models. Due to the sudden and rapid spread of MPXV during the last epidemic, it is fundamental to investigate the neurological sequelae associated to Mpox, and to identify new therapeutic strategies to counteract this virus and other poxviruses.

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Keyword: *MPXV, Neurotropism, Neuroinflammation, Human pluripotent stem cells*

#317 Amyloid Precursor Protein and Tau Peptide Mixture or Linked Peptides Ameliorate Cognition in an Alzheimer's Disease Animal Model

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The major proteins involved in Alzheimer's disease (AD) are amyloid precursor protein (APP) and Tau protein which can bind to each other. We demonstrate, that APP1 (390-412) and Tau1 (19-34) peptides, in a mixture, are able to inhibit in vitro the interaction between APP and Tau proteins. The APP1 and Tau1 peptides linked together, by a flexible or rigid linker, are able to inhibit the interaction between APP and Tau proteins as well. Nasal administration of biotin-labelled Flex peptide, to 5xFAD mice, for two weeks, indicate localization of the peptide around and close to plaques in the hippocampus area. In vivo studies were performed in an AD mouse model 5xFAD transgenic (Tg) mice as well as in 5xFAD crossed with Tau Tg. These mice exhibit plaque load and mild cognitive decline starting at 4 months of age, were nasally treated with Mix, Flexible or Rigid linked peptides. Treatment decreased amyloid plaque burden as well as the deterioration of cognitive functions. The effect of treatment was significant when initiated at the age of three to five months, before severe cognitive deficiency is evident, or when such deficiency is already observed before treatment started. The nasal treated mice demonstrate a cognitive ability not significantly different from the non-Tg littermate control. Testing the effect of the flexible peptide by gavage feeding, on the cognitive function of 5xFAD Tg mice, demonstrate that feeding as well as nasal treatment improves significantly the cognitive ability of Tg mice compared to control PBS treated mice.

#320 Homeostasis of polymorphonuclear neutrophil cells in drug-resistant epilepsy patients

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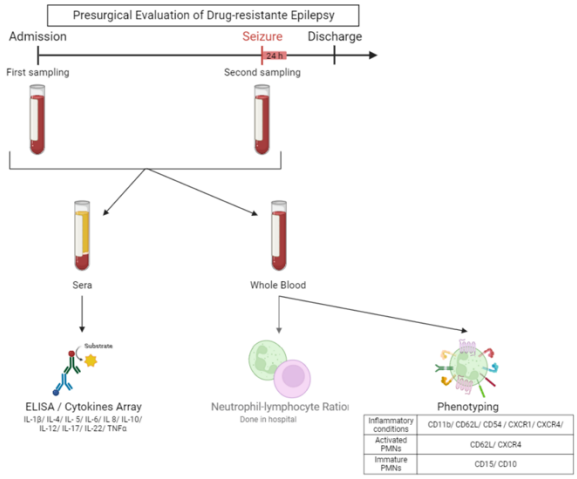
Epilepsy is a neurological disorder characterized by recurrent and spontaneous seizures associated with tissue damage and inflammatory processes. Neuroinflammation, recognized to contribute to neuronal hyperexcitability, could be a consequence of peripheral inflammation, notably through the migration of peripheral leukocyte across the blood-brain barrier (BBB). Polymorphonuclear neutrophils cells (PMNs), key cells of innate immunity, are also important mediators of vascular and tissue damage induced by inflammation. In epilepsy, an increase of peripheral PMNs is associated with elevated levels of IL-8 (Interleukin-8), major cytokine that attracts and activates PMNS. However, characterization of PMNs phenotype remains poorly understood in epilepsy.

Blood samples of epileptic patients were collected during their presurgical evaluation, at their admission, within 24 hours post seizures, and before surgery. Systemic inflammation was evaluated for i) the levels of pro-inflammatory cytokines in patient sera using SIMOA technology (Interleukin-1b, 4, 5, 6, 8, 10, 12, 17, 22 and tumor necrosis factor (TNF), and ii), the ratio neutrophil-lymphocyte (NLR) in blood. PMNs phenotype was assessed by flow cytometry in whole blood, focusing on specific membrane marker regulated in inflammatory conditions (CD11b, CD62L, CD54, CXCR1, CXCR4), specific marker of immature PMNs (CD10, CD15), and activated PMNs (CXCR4, CD62L).



Our studies found a chronic systemic inflammation in patient with a significant increase of IL-6 and IL-8 in sera, along with an increase of NLR. Flow cytometry revealed a shedding of a majority of membrane markers, characterizing activated and/or immature PMNs. Activated (CXCR4+, CD62Llow) and hyperactivated (CXCR4high, CD62Llow) PMNs were found significantly higher, and positively correlated with IL-6, IL-8 and TNFalpha. Immature PMNS (CD10-CD15+) were also found elevated in blood of epileptic patient, however no correlation with systemic inflammatory state was observed.

This project is the first to assess the phenotype of PMNs in epileptic patients and show an abnormal activation of PMNs. Interestingly, activated PMNs have been linked to BBB disruption in other neuropathologies, while BBB disruption was shown to be linked to epileptogenesis. Further understanding mechanism of PMNs activation in epilepsy could lead to new perspectives in the development of innovative immunotherapy strategies.

Keyword: *Epilepsy, Neutrophil, Chronic inflammation*

#333 Immunomodulatory effect of free and AOT/(PLL/PGA)2-g-PEG nanoparticles loaded carnosic acid in lipopolysaccharide treated organotypic hippocampal cultures

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Age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease belong to significant health problems in the contemporary world. Even worse, the available treatment is mainly symptomatic, and its efficacy is limited to the early stages of the disease progression. A large body of evidence has accumulated that neuroinflammation plays a pivotal role in the background of these devastating brain disorders, suggesting that some new anti-inflammatory agents and /or pro-resolving mediators could at least slow down the neurodegenerative processes. However, since the blood–brain barrier (BBB) prevents the brain uptake of most pharmaceuticals, it is necessary to design an efficient delivery of the putative neuroprotective drugs to the central nervous system and maintain their therapeutic concentration in the brain tissue. To this end, some nanoparticles can cross BBB and suitable properties for drug delivery, such as controlled drug release and targeting efficiency.

Therefore, this study aimed to establish and evaluate the ability of AOT/(PLL/PGA)2-g-PEG nanoparticles loaded with rhodamine as a fluorescent marker to cross BBB in an experimental in vitro model: hCMEC/D3 human

cell line. Next, we estimated the neuroprotective potential of carnosic acid (CA) encapsulated in AOT nanocarriers in organotypic hippocampal cultures (OHCs) exposed to a nonspecific immune system activator - lipopolysaccharide (LPS).

The results indicated that the designed nanocarriers could cross BBB in a time-dependent manner and that the maximum fluorescence signal was obtained after 48 hours of exposure. Moreover, we revealed that LPS stimulation increased pro- and anti-inflammatory cytokine synthesis (IL-1beta, TNFalpha, IL-18, IL-6, IL-4, IL-10) and microglia markers (CD68, CD40) expression in OHCs. Administration of free CA decreased the LPS-induced hippocampal damage as measured by LDH test and NO synthesis. Pre-treatment with CA alone at concentrations 5, 10, and 25 mikroM affected the effects of LPS as evidenced by attenuation of some cytokine levels (e.g., IL-1beta, IL-4). Of note, the immunomodulatory effects of CA encapsulated in the tested nanocarriers were preserved.

In conclusion, the obtained data indicate that AOT/(PLL/PGA)2-g-PEG nanoparticles can be efficient nanocarriers of neuroprotective drugs to the central nervous system. Moreover, this study demonstrated the modulatory properties of CA. It showed that this compound could be a suitable component of nanotheranostics in developing modern neuroprotective strategies based on the resolution of inflammation.

This research was supported by grant no. 2019/34/H/ST5/00578 from the National Science Centre, Poland.

#346 Myeloid Dickkopf-1 fuels neurovascular and neuroimmune alterations in ischemic stroke

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Introduction

Stroke constitutes a major cause of death and disability. We have previously shown that ischemic stroke deregulates the canonical Wnt pathway, which plays key roles in controlling neurovascular functions. The circulating levels of dickkopf-1 (DKK1), a major endogenous inhibitor of the pathway, are elevated in stroke patients and correlated with poor prognosis. How DKK1 worsens stroke outcomes remains unknown.

Methods and Objectives

Our study aims to elucidate the role of DKK1 in ischemic stroke pathobiology and therapy, remains unknown. For this purpose, we used a novel conditional mouse model that enable spatiotemporal induction of DKK1 in an appropriate tissue-specific manner (iDKK1 mice). Mice were subjected to ischemic stroke through middle cerebral artery occlusion (MCAo) and state-of-the-art approaches we applied to investigate the impact of DKK1 induction of neurovascular functions. Furthermore, pharmacological approaches were used to neutralize DKK1's biological activity.

Results

Our results demonstrated that DKK1 is de novo expressed at the lesion site after ischemic stroke. DKK1 induction prior to stroke onset exacerbated infarct and oedema sizes as well as aggravated motor deficits after stroke. Furthermore, it increased neuronal degeneration and altered neurogenesis, neuronal maturation as well as neuronal activity. DKK1 induction impaired tissue

vascularization and regional cerebral blood flow (CBF), as well as dysregulated the neuroinflammatory responses and glial scar organization. Interestingly, delayed DKK1 induction after stroke onset attenuated long-term restoratives processes and motor recovery. Using mRNA-Seq, we outlined transcriptomic signatures at the lesion site that were associated with a chronic neuroinflammation and psychiatric disorders. Indeed, DKK1 delayed induction increased anxiety-like behaviors in mice. Finally, pharmacological neutralization of DKK1's biological activity improved structural and functional recovery after stroke. Finally, we provide evidence that DKK1 is virtually absent in the brain under normal conditions, and its de novo expression at the lesion site is associated with infiltrating immune cells.

Conclusion

Herein, we demonstrated that DKK1 plays a central role in mediating neurovascular and neuroimmune deregulation after ischemic stroke. Our results indicate that its neutralization constitutes a clinically relevant approach to promote brain repair and neurological recovery after ischemic stroke.

Keyword: *DKK1, Stroke, Wnt pathway, neuroinflammation*

#365 Sensory neuron-derived miR-21-5p promotes neuropathic allodynia through inhibition of TGFB-related pathway in macrophages.

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Neuropathic pain represents a significant therapeutic challenge, with current treatments often providing inadequate analgesia. Consequently, there is a pressing need to enhance our understanding of the underlying mechanisms of chronic pain and identify innovative targets. In peripheral nerve injury models of neuropathic pain, sensory neuron cell bodies in the dorsal root ganglia (DRG) up-regulate miR-21 for up to 14 days after injury. We have shown that activated nociceptive neurons release miR-21 encapsulated in exosomes which are engulfed by macrophages that are in contact with neurons. This transfer process promotes a pro-inflammatory phenotype in macrophages, which contributes to sensitization of nociceptive neurons that leads to allodynia.

We have recently shown that neuron-derived miR-21 transfer to macrophages maintains a pro-inflammatory phenotype by suppressing TGFB anti-inflammatory pathway. Indeed, miR-21 conditional deletion in DRG neurons (miR-21 cKO mice) is associated with significant attenuation of pain-like behaviour (allodynia) at day 7 after spared nerve injury (SNI) via the activation of TGF-beta pathway in macrophages and polarisation towards an anti-nociceptive M2-like phenotype. Furthermore, on one hand intrathecal injection of a TGF-Beta receptor antagonist restored neuropathic allodynia in miR-21 cKO mice, on the other hand injection of macrophages transfected with miR-21 antagomir attenuated neuropathic allodynia in WT mice.

Since miR-21 is still up-regulated in DRG at 14 days after injury when neuropathic allodynia persists, we considered this time point and observed less macrophage accumulation in miR-21 cKO DRG, and polarisation towards an anti-nociceptive M2-like phenotype via up-regulation of CD206. Moreover, we performed single cell RNA-seq and identified multiple dysregulated genes both in sensory neurons and macrophages. Therefore, we are currently testing the hypothesis that miR-21 affects multiple targets within DRG sensory neurons, consequently contributing to the maintenance of M1-like

phenotype of macrophages and persistence of neuropathic allodynia.

Overall, our study highlights the significance of miR-21 in modulating DRG neurons and macrophages in neuropathic pain and provides insights into the potential development of novel therapeutic strategies targeting miR-21 to alleviate neuropathic pain conditions.

Keyword: *miR-21, macrophages, DRG, Neuropathic pain*

#375 The Complement System in an Argentinian Cohort of Myasthenia Gravis Patients: Evaluation of Soluble Proteins and Membrane-Bound Regulators

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Myasthenia gravis (MG) is an autoimmune disease characterized by autoantibody-mediated dysfunction at the neuromuscular junction. These autoantibodies primarily target the acetylcholine receptor, triggering activation of the complement system's classical pathway, which leads to muscle fibre destruction. The complement system consists of over 50 proteins, many of which are involved in regulating the activation sequence. Although various complement regulator proteins (CRPs) have been evaluated in animal models, their study in MG patients remains limited.

To address this gap, we assessed the expression of three membrane-bound CRPs, namely CD59, CD46, and CD55, on white blood cells (WBCs) from 15 MG patients and 8 healthy controls (HCs). The severity of MG was assessed and recorded using ADL and MGC clinical scores. Blood samples were collected in heparinized tubes and processed 24 hours later. After red blood cell lysis, WBCs were incubated with monoclonal antibodies against CD59-FITC, CD55-APC, and CD46-PE for 30 minutes at room temperature. Flow cytometry analysis was conducted to measure the expression of these markers on the WBCs' granulocyte subpopulation, which is known to exhibit the highest CRP expression, and represents the most abundant cell population among WBCs. Additionally, plasma levels of complement C3, C4 and C5a proteins were measured in the samples.

Our findings revealed a lower mean fluorescence intensity (MFI) for all three CRPs on granulocytes in MG patients compared to HCs. However, only CD46 expression (MFI) exhibited a statistically significant difference compared to HCs (MG: 5649 ± 1628 vs HCs: 7680 ± 1591, p=0.009). Preliminary analysis of this small patient group did not identify a statistically significant correlation between CRP expression and MG severity nor between CRP expression and C3, C4 and C5a plasma levels.
In conclusion, this study suggests that diminished expression of these three CRPs, particularly CD46, may contribute to increased susceptibility to complement-mediated damage in MG patients. However, further investigations involving a larger participant cohort are necessary to validate these findings.

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Keyword: *complement system, myasthenia gravis, membrane-bound complement regulator proteins, CD46, C5a*

#376 HNF4-alfa, SP1 and c-myc are master regulators of CNS autoimmunity

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system (CNS), characterized by heterogeneity in the symptoms, disease course, and outcomes. Most of the currently approved therapies have shown great efficacy in treating the relapsing-remitting (RR) form of MS but not the progressive forms. In this study, we searched for potential therapeutic targets shared among all phases and forms of MS.

We performed network biology studies of transcriptomes from peripheral blood mononuclear cells (PBMC) from healthy subjects and patients with Clinically Isolated Syndrome (CIS) or definite MS (RR, Secondary Progressive and Primary Progressive courses) and found HNF4-alfa, SP1 and c-myc as three master transcription factor (TF) hubs regulating differential expression at all MS stages. To unravel the role of these TFs in immunity we measured nuclear protein levels of the three targets in PBMC from healthy subjects and observed that HNF4-alfa, SP1 and c-myc were expressed in human T cells and monocytes and upregulated in vitro upon activation or exposure to environmental MS risk factors (cigarette smoke and low vitamin D levels). Notably, HNF4-alfa and SP1 but not c-myc were enriched in MS blood cells compared with cells from healthy individuals, and super-resolution microscopy confirmed major TF and colocalization in MS cells, suggesting TF complex formation.

We then checked the effect of TF inhibition in the experimental autoimmune encephalomyelitis (EAE) model of MS. Importantly, administration of HNF4-alfa, SP1 or c-myc inhibitors in a therapeutic setup, either alone or in combination, significantly ameliorated disease expression and reduced ex vivo encephalitogenic T cell responses, without showing a synergistic effect.

Our research identified key transcriptional regulators shared by all stages of MS, which may represent attractive therapeutic targets also for progressive MS.

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#377 T helper-released extracellular DNAs promote CNS autoimmunity

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Regulated extrusion of genetic material is primarily considered an inflammatory mechanism deployed by innate immune cells and called ETosis, which contributes to protection against infections or exacerbation of tissue damage under sterile inflammatory conditions. Recently, we have shown that mouse naïve CD4+ T cells release threads of oxidized DNA on activation, which were named T helper-released extracellular DNAs (ThREDs). These DNA fibers, mainly of mitochondrial origin, intrinsically sustain the effector functions of naïve CD4+ T cells by promoting the production of pro-inflammatory cytokines. Here, we extended our analysis to human cells and found that polyclonally-activated human CD4+ T lymphocytes release ThREDs forming web-like structures among clusters of cells. These DNA fibers can be associated with the transcription factor A mitochondria (TFAM) and partially resemble extracellular traps released during ETosis, as they are positive for a typical marker of this phenomenon such as citrullinated histone H3 (CitH3). Human and mouse CD4+ T lymphocytes express peptidyl arginine deiminase (PAD)4, a key enzyme mediating histone citrullination and ETosis, both at the transcriptional and protein levels. Exposure of human and mouse CD4+ T cells to a highly-selective PAD4 inhibitor dampens the release of CitH3+ ThREDs and decreases the production of pro-inflammatory cytokines. Clusters of CitH3-positive T helper cells accumulate in the spinal cord of mice during the acute phase of relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE). Administration of a PAD4 inhibitor after the onset of the first signs of R-EAE significantly improves clinical manifestations, reduces demyelination and immune cell infiltration into the spinal cord, and attenuates the pro-inflammatory potential of myelin-reactive CD4+ T cells. This study adds novel findings supporting the involvement of ThREDs in CNS autoimmunity.

Keyword: *extracellular DNA, CD4+ T cells, EAE, sterile inflammation*

#386 Neuroinflammation and basal ganglia circuit dynamics: a study on the effects of acute peripheral inflammation on dopaminergic neurons

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**Equally contributed.*

Background. The basal ganglia (BG) circuit is a neuronal network mediating locomotor activity and movement execution, emotional states, social behavior, reward, and motivation. The activity of the nucleus striatum, the input station of the circuit, is strongly regulated by dopamine (DA)-releasing midbrain neurons (1). An

abnormal activation of the immune system (IS) characterizes the pathogenesis of both psychiatric and neurological disorders and is known to influence neuronal network functioning in the cerebral cortex (2,3). To date, the influence of IS activation on DA-releasing cells and BG circuit dynamics is poorly understood. **Material and methods.** Electrophysiological analyses of midbrain DA neurons activity and striatal synaptic plasticity were performed in lipopolysaccharide-(LPS) treated animals, 24 hours after treatment, and in control animals. Local inflammation patterns and dopamine neuron markers were assessed through immunohistochemistry. **Results.** Acute peripheral inflammation increased midbrain DA neurons' firing rate and excitability state and was associated with impaired striatal synaptic plasticity. Immunohistochemical analysis showed an unaltered number of midbrains DA cells, while it showed increased activation of microglial, but not astroglial markers at the level of the substantia nigra and no changes in inflammatory markers at the level of the nucleus striatum. **Discussion.** The obtained data suggest that acute systemic inflammation results in functional alterations of the BG circuit at multiple levels. **Conclusions**. The involvement of DA neurons after IS activation could underlie cognitive and psychiatric symptoms in neuroinflammatory brain disorders.

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Keyword: *dopamine transmission, peripheral inflammation, synaptic plasticity*

Novel approaches for neuroimmunologists

#60 CanProCo Study; Determining Technical Parameters for Large Scale Single-cell RNA Sequencing of Multiple Sclerosis Patients

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Background: The heterogenic clinical presentation and unpredictable disease trajectory of multiple sclerosis (MS) remains a prevalent challenge for patient care. Predictive and prognostic biomarkers are lacking, but much needed. The Canadian Prospective Cohort to Understand Progression in MS (CanProCo) study involves collection of biological, radiological, and clinical data from 1000 MS patients early in the disease course, with blood collected annually for

five years. Single-cell RNA sequencing will be performed on 250 MS patient peripheral blood mononuclear cells (PBMCs) prior to treatment initiation. **Objective**: Transcriptomic profiles will be integrated and compared across two single-cell sequencing platforms, and the number of sequenced cells and depth of sequencing (reads/cell) will be analyzed to determine ideal sequencing parameters for the PBMCs from 200 CanProCo patients. **Method:** Single cell libraries were generated, and deep sequencing (70k reads/cell) was performed on one relapsing-remitting MS patient (18,627 cells), one sex-matched healthy control (14,148 cells) on BD Rhapsody. The same MS patient's PBMCs and one healthy control (7,828 cells) were sequenced on the 10X Genomics platform (8,968 cells) as well. **Results:** Unbiased and biased clustering identified 12 putative immune cell clusters. The proportion of each cell type and top ten differentially expressed genes in each cell cluster was similar across both sequencing platforms. By down-sampling the number of cells, the proportion of each cell cluster remained similar, even at 500 cells. T cells were extracted and unbiasedly re-clustered to identify nine unique T cell clusters. Rare T cell populations, like gamma-delta T cells, were still detectable at 8,968 cells. Despite the variation in sequencing depth (12k vs 70k reads/cell), the number of unique genes and the transcriptomic profile of putative cell types were similar. Comparison of the number of transcripts and unique mapped genes showed that the level of sequencing saturation varied across cell types, which highlighted an important consideration when determining optimal sequencing depths for specific cells of interest. **Conclusion:** These preliminary data have guided sequencing plans for CanProCo patient PBMCs. Single cell transcriptomic profiles of MS patients will be eventually analyzed alongside extensive clinical, imaging, and demographic data. Understanding the transcriptomic profile at a single-cell resolution will elucidate gene signatures which may dictate disease trajectory, treatment response and reveal novel prognostic biomarkers to improve clinical care of people with MS.

Keyword: *Single-cell sequencing, Transcriptomics, Multiple sclerosis*

#103 Glial responses to implantable neural devices

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Neurostimulation is a commonly used therapeutic treatment for neurological and psychiatric disorders that requires neural devices to be implanted in patient brains for several years. For example, deep brain stimulation is an FDA-approved procedure proven to provide durable symptomatic relief in Parkinson's Disease. However, foreign body response (FBR) and encapsulation can deteriorate device efficiency and overall performance over time, thus diminishing the therapeutic potential of neurostimulation. The impact of chronic neurostimulation and indwelling implant on changes in glial reactivity across brain regions and how they affect biocompatibility and long-term therapeutic benefits has not been fully examined. To date, most studies quantify cellular response to neuronal devices using measurements of image fluorescence intensity. Quantitative approaches that provide reproducible data across studies, such as cell density, morphology, and location are lacking. To characterize the heterogeneity of glial responses to implantation trauma, indwelling implants, and active

electrodes, we studied the changes to cortical and hippocampal IBA1-microglia and GFAP-astrocytes at 2 and 6 weeks after insertion trauma and implantation of identical silicon or polyimide passive multi-shank microelectrodes in the mouse brain. We hypothesized that neurotrauma would be resolved and any persisting FBR at 6 weeks (sub-chronic) after electrode implantation will be different across brain regions and material. To address the changes in glial reactivity independent of immunofluorescence measurements, we developed an automated cell identification pipeline to count IBA1-microglial cells and GFAP-astrocytes in a 150-µm radius around microelectrode shanks or insertion sites. R was used to process vector coordinates of detected cells to measure intercellular distances and distance of each cell to the insertion or implantation sites. At 6 weeks, we found no difference in IBA1-microglia and GFAP-astrocytes densities after insertion trauma in both cortex and hippocampus compared to healthy tissue. However, the increase in hippocampal IBA1-microglia and GFAP-astrocytes densities at 6 weeks after microelectrode implantation compared to control remained significant. Detailed analyses revealed a more complex pattern of cellular distribution and glial morphologies around the insertion site and implants, such as 10–27% smaller glial intercellular distances at sub-chronic timepoint of passive electrode implant. Our results surpass earlier findings by revealing complex IBA1-microglial and GFAP-astrocytic density, distribution, intercellular distances, and morphologies at acute and sub-chronic implantation timepoints in different brain regions. Combined with transcriptomic analysis, our comprehensive characterization of glial responses will lead to more detailed assessment of tissue response to neural devices and guide further strategies that target glial cells to improve long-term biocompatibility of active neural devices.

See Abstract “Autogliji: rapid automated pipeline for cell morphological analysis”

Keyword: *astrocytes, microglia, heterogeneity, neurotechnologies, foreign body response*

#104 Autogliji: rapid automated pipeline for cell morphological analysis

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Changes in morphological features of glial cells are strong indicators of their response to their environment across health and disease conditions. However, current approaches to analyze morphology either involve time-consuming manual measurements of morphometric features and are prone to selection bias, or require expensive commercial software packages that limit their broader reach to all user groups. To address these limitations, we developed a semi-automated pipeline for high throughput cell morphological analysis using the open-source image analysis software ImageJ, also known as Fiji. Our pipeline is optimized for two-dimensional (2D) histological or immunofluorescence images, as well as Z-projected image volumes. Upon segmenting individual cells within an image, our program reconstructs the shape of the cells to generate measurements such as soma area, whole cell area, cell circularity, total number of cellular

processes, number of process junctions, number of process endpoints, as well as average and maximum lengths of processes. Preliminary data from beta testers (N=20) with different levels of background in cell biology support our argument of user-induced biases in manual cell morphological analyses. Large deviations in results from manual cell tracing were recorded in contrast to using Autogliji during controlled experiments to measure 3 ramified microglial cells. An average of 40 minutes was required for manual efforts in contrast to 5 minutes with our intuitive pipeline. Autogliji may acquire accurate and reproducible measurements of morphological features for up to 99 cells per image within minutes. With this high throughput, we could rapidly assess similarities and heterogeneity of astrocytes, microglia and other tissue-resident macrophages through their morphologies under various conditions and from different animal species. Overall, our pipeline allows for efficient large-scale data collection for the holistic study of cell biology across homeostasis and pathology.

See Abstract "Glial responses to implantable neural devices"

Keyword: *glia, heterogeneity, morphology, batch analysis*

#122 Pathophysiological Effects of Microgravity and Weightlessness; A Review of the Literature

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OBJECTIVE: Numerous studies have demonstrated that weightlessness, microgravity or zero gravity, has physiological and pathological effects on multiple organ systems in humans and animals. Clinicopathologic changes that impact the cardiovascular, skeletal, immune, endocrine and nervous systems have been described in the biomedical literature. Zero gravity environments induce a number of transient pathophysiological effects in individuals on short-term space flight and extended space station duty. The objective of this study was to review the growing scientific data on the effects of microgravity, and in combination with space radiation, on physiology and pathophysiology.
METHODS: We conducted a systematic literature search through the Saint James School of Medicine Library and the Kean University Nancy Thompson Library using PubMed, ProQuest and EBSCO electronic databases. The text words, "microgravity", "weightlessness", and "spaceflight", with the use of the Boolean operator "AND" the terms "respiratory system", "cardiovascular", "heart", "brain", "nervous system", "digestion", "reproduction", "hormones", "endocrine", "immune system", "immunological", "muscle", "musculoskeletal system", "bone", "skin", and "integumentary system" were used to identify relevant studies discussing the physiologic changes and pathologic manifestations following exposure to short term microgravity and longterm spaceflight. Inclusion criteria were the following: 1) must be a scholarly or peer-reviewed source, 2) a relevant article within the last 12 years, and 3) articles published in the English language only. Outcome measures included changes in tissues and cells in the risk groups.
RESULTS: 45 studies were included on both human cosmonauts, astronauts, animal models and cell lines. Weightlessness effects on the cardiovascular system included decreased heart size, increased incidence of negative EKG events, arrhythmias, and intimal thickenning in blood vessel. In the skeletal system, exposure to weightlessness in combination with radiation

decreased bone minerals, decreased bone density, impaired osteoclast differentiation and decreased osteoblasts, thus, accelerating bone degeneration and diminishing bone size. Microgravity induced muscle atrophy and decreased volume of transverse abdominus and multifudus muscle at L5, while resulting in hypertrophy of internal oblique muscles. Immune function was also adversely impacted by weightlessness, which impaired T-cell-mediated responses, increases levels of harmful reactive oxygen species (ROS), impaired macrophage cytoskeletal structure, CD68 and MHC-II surface expression, reduced lymphocyte activation in response to mitogenic stimuli, reduced cytotoxicity of natural killer cells, reactivation of latent viruses and reduced delayed-type hypersensitivity reactions in response to common recall antigens. In the reproductive system, microgravity damaged sperm DNA, induced spermatogenic cell apoptosis, diminished testicular weight and tubular diameter. Endocrinological effects of weightlessness included increased levels of epinephrine, decreased levels of cortisol, morphological and functional changes within the thyroid gland. Neurologic impairment included increased glioma cell death by apoptosis, decreased glioma cell migration, increased disorganization of microtubules, decreased brain activity in the left cerebellum, paracentral, anterior cingulate, superior frontal gyrus, and limbic lobe, right lingual, post-central, and middle temporal gyri. In addition, changes in the gut microbiome were noted.

CONCLUSION: Some of the microgravity-induced physiologic changes are reversible. However, there are concerns that long-term exposure to zero-gravity conditions could have long-term adverse consequences leading to chronic pathophysiologic patterns. Notably, exposure to microgravity induces physiological changes consisent with accelerated aging. The findings thus far have been derived from a small number of individuals. Further studies are warranted to evaluate large populations of individuals, especially that space tourism is on the horizon and may pose new medical risks for members of the general public with unscreened health status. These risk factors must be studied in order to allow for the successful progression of long-term space flight missions and space tourism.

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Keyword: *microgravity, weightlessness, zero-gravity, immune suppression, bone density*

#123 Deep Learning-based assessment of locomotion in experimental autoimmune encephalomyelitis in an accurate, unbiased method to evaluate clinical outcome.

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Multiple Sclerosis (MS) is the most prevalent neurodegenerative disease in young adults, affecting an estimated 2.8 million people worldwide. Hallmarks of MS include oligodendrocyte cell death, demyelination, peripheral immune cell infiltration and axonal damage causing motor impairment. Pre-clinical studies to investigate MS pathophysiology largely rely on the experimental autoimmune encephalomyelitis (EAE) model, where immunization with myelin-derived peptides leads to a T cell-mediated response resulting in a clinical phenotype that recapitulates MS symptoms. The gold standard for measuring locomotor dysfunction associated with EAE is a clinical scoring system typically graded from 0 to 6. This, however, has limitations. Beside the variability and subjectivity inherent to any investigator-dependent behavioral test, the major pitfall of this scoring system is its inability to detect subtle changes that occur in the very early stages of disease, when obvious locomotor deficits have yet to appear. Indeed, it is only 10 to 15 days post immunization (dpi) that symptoms can be scored, but the pathological processes responsible for disease development are already in full effect. Thus, developing sensitive and quantitative methods for evaluating locomotor behavior in mice, particularly in the early stages when therapeutic interventions could be most effective, is an unmet need that we aim to address.

To this end, we developed the MotorBox, a marker-less kinematic assessment system based on machine learning algorithms using the DeepLabCut open-source toolbox. With this method, we determined that mice in the early stage of EAE (10 dpi), considered pre-symptomatic based on their clinical score of 0, already exhibit altered coordination, gait, balance, agility, stride length, velocity and synchronicity compared to naïve mice. Studies are ongoing to establish whether these early alterations may predict EAE severity at the acute phase.

In conclusion, our MotorBox approach provides sensitive and unbiased metrics of locomotor function that are invaluable to accurately assess early clinical outcomes of EAE otherwise not detected by the classic clinical scoring system. This will help inform the timing of therapeutic interventions, predict clinical outcomes and allow for more precise analysis of EAE progression.

Keyword: *Machine Learning, Multiple Sclerosis, Kinematics*

#138 Elevated neurocan in intracerebral hemorrhage lesions impairs oligodendrogenesis

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Background Intracerebral hemorrhage (ICH) is the predominant type of hemorrhagic stroke with high mortality and disability. In other neurological conditions, the deposition of extracellular matrix (ECM) molecules is a prominent obstacle for regenerative processes and an enhancer of neuroinflammation. Whether ECM molecules alter in composition after ICH, which ECM members may inhibit repair, and how to overcome inhibitory phenotype of ECM remain unknown in hemorrhagic stroke.

Methods The collagenase-induced ICH mouse model was investigated for expression of ECM members and oligodendrocyte lineage cells by immunofluorescence microscopy. An autopsied human ICH specimen was examined for neurocan expression. Confocal image z-stacks were analyzed with Imaris 3D to assess the association of immune cells and ECM molecules. Sections from a mouse model of multiple sclerosis were used as disease controls. Tissue culture was employed to examine the roles of ECM members on oligodendrocyte precursor cells (OPCs). Western blot was applied to detect ECM proteins. The collagenase-induced ICH mice were treated with Ac-4, 4-diF-N-acetylglucosamine ("difluorosamine") in attempts to reduce neurocan content. Behavioral tests were conducted at multiple time points.

Results Amongst the lectican chondroitin sulphate proteoglycan (CSPG) members, neurocan but not aggrecan, versican-V1 and versican-V2 was prominently expressed in perihematomal tissue and lesion core compared to the contralateral area in murine ICH. Fibrinogen, fibronectin and heparan sulphate proteoglycan (HSPG) were also elevated after murine ICH while thrombospondin was not. Confocal microscopy with Imaris 3D rendering co-localized neurocan, fibrinogen, fibronectin and HSPG molecules to Iba1$^+$ microglia/macrophages or GFAP$^+$ astrocytes. Marked differentiation from the multiple sclerosis model was observed, the latter with high versican-V1 and negligible neurocan. In culture, purified neurocan inhibited adhesion and process outgrowth of OPCs, which are early steps in myelination in vivo. The prominent expression of neurocan in murine ICH was corroborated in human ICH sections. Difluorosamine treatment markedly reduced neurocan levels at both perihematomal area and lesion core, and increased the number of Olig2$^+$ PDGFRα$^+$precursors and Olig2$^+$ CC1$^+$ mature oligodendrocytes at perihematomal area. Treated mice also had improved functional recovery following ICH.

Conclusion ICH caused distinct alterations in ECM molecules. Amongst CSPG members, neurocan was selectively upregulated in both murine and human ICH. In tissue culture, neurocan impeded the properties of oligodendrocyte lineage cells. The CSPG-reducing drug, difluorosamine,

enhanced oligodendrogenesis and promoted recovery after ICH.

Keyword: *intracerebral hemorrhage, oligodendrogenesis, neuroinflammation, extracellular matrix*

#288 A multi-modular approach to characterize multiple sclerosis brain pathology in rapid autopsy brain tissue

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Background: Detailed characterization of immune cells in multiple sclerosis (MS) brain lesions is commonly performed by immunofluorescence (IF). This technique is limited by the reagent availability (e.g. antibodies) and the number of concomitant immuno-detections to characterize immune cells and CNS resident cells. Therefore, the need for other techniques to further explore the profile of these cells is high.

Aims & Objectives: To design a multimodal method to extensively characterize the phenotype of infiltrating immune cells and CNS resident cells using single-cell RNA sequencing (scRNAseq), spatial RNA sequencing (spRNAseq), bulk RNAseq, confocal microscopy, flow cytometry according to the type of MS brain lesions as assessed by histology and histochemistry.

Methods: Brain tissue with short post-mortem delays from patients with MS (n=4) and other neurological disease (OND, n=6) was cut into coronal slabs, and demyelinated areas (lesions) or normal-appearing tissue were dissected from grey (NAGM) and white matter (NAWM) (n>100). Various brain regions were similarly sampled between MS and OND. One-third of the lesion was directly frozen for lesion characterization (pre-active, active, mixed active/inactive, and inactive). The rest was processed for analysis by scRNAseq, bulkRNAseq, or flow cytometry. Confocal microscopy and spRNAseq analyses were used to corroborate findings from other modalities.

Results: Bulk RNAseq analysis identified transcriptomic signatures associated with lesions as compared to NAWM. Periventricular lesions were significantly different from parenchymal white matter lesions. Using flow cytometry, confocal microscopy, scRNAseq and spRNAseq we characterized T and B cell subsets, macrophages, microglia, dendritic cells, astrocytes, and oligodendrocytes in single lesions. WGCNA (weighted gene correlation network analysis) on the bulk RNAseq revealed correlated modules, which were linked back to scRNAseq to identify cell-specific signatures. Furthermore, we identified CD6 as a molecule of interest on infiltrating $CD4^+$ and $CD8^+$ T cells

preferentially enriched in mixed active-inactive (smoldering) white matter lesions.

Conclusion: The combination of complementary modalities simultaneously analysing single MS lesions allows to characterize multiple types of immune and CNS resident cells. This multidimensional characterisation leads to an unprecedented view into the disease processes that underlie lesion formation and evolution.

Keyword: *Multiple Sclerosis, single cell RNA-seq, spatial RNA-seq, Multiple Sclerosis Lesion Pathology, Rapid autopsy brain tissue*

#324 Elucidating the impact of CSF1R mutations on microglia development and function using patient-derived iPSCs

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Previous research has shown that specialized glial cells in the brain, called microglia, regulate neurogenesis, promote neuronal survival, and support neurodevelopmental processes. A key microglial receptor, colony-stimulating factor 1 receptor (CSF1R), modulates these critical functions of microglia, but in a rare neurodegenerative disease called adult leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), heterozygous pathogenic variants lead to progressive white matter disease. In this disorder, microglial activation is driven by the dysregulation of CSF1R expression, thereby inducing a state of increased inflammation, contributing to neurodegeneration and white matter loss. Since CSF1R is an important receptor for microglia, and since induce pluripotent stem cells (iPSC)-derived microglia protocols rely on CSF1R ligand binding, cells with mutations in this gene do not proliferate or differentiate well using standard culture methods, which complicates the process of generating an in vitro ALSP model. To develop a novel ALSP model, we reprogrammed peripheral blood mononuclear cells (PBMCs) from ALSP patients into iPSCs to later differentiate them into microglia by varying the culture conditions. We identified growth conditions in which microglia can be differentiated from iPSCs, but survival and differentiation states were altered in mutant cells compared with controls. In parallel, we are characterizing these cells by assessing how phagocytosis, cytokine release, migration, and adhesion vary between control and mutated lines. Understanding how variants in the CSF1R gene affect the development and function of microglia will allow us to lay the foundation for identifying potential therapeutic options for treating ALSP and generate a robust disease model that will contribute to our understanding of various microglia-mediated white matter diseases.

Keyword: *Microglia, CSF1R, ALSP*