UAlberta MS Centre Research Symposium

Emerging Leaders in MS Research in Canada

THERE IS ONLY ONE CURE FOR MULTIPLE SCLEROSIS: RESEARCH

Friday, May 5, 2023, Telus Centre, North Campus
08:00—17:15

UNIVERSITY OF ALBERTA

UNIVERSITY of ALBERTA
MULTIPLE SCLEROSIS CENTRE

NEUROSCIENCE AND MENTAL HEALTH INSTITUTE | University of Alberta
Brain Health and Injury Research

Event Itinerary

08:00	Continental Breakfast, Atrium
08:45	Welcome & Introduction, Bill Flanagan UAlberta President
09:00	Speaker: Dr. Anastassia Voronova, University of Alberta
09:40	Speaker: Dr. Shannon Kolind, University of British Columbia
10:20	Break, Atrium
10:35`	Speaker: Dr. Hedwich Kuipers, University of Calgary
11:15	Speaker: Dr. Lawrence Steinman, Stanford University
12:15	Lunch/Trainee Posters, Atrium
13:30	Trainee Presentations, T. MacKeigan and B. Hammond Theatre
14:00	Speaker: Dr. Jason Plemel, University of Alberta
14:40	Speaker: Dr. Anna Taylor, University of Alberta
15:20	Break, Atrium
15:35	Speaker: Dr. Catherine Larochelle, Université de Montréal
16:15	Question-and-Answer session, moderated by Nicole Sullivan of MS Canada
17:00	Closing remarks

Welcome

Welcome to the 5th annual University of Alberta MS Research Symposium!

The understanding of multiple sclerosis (MS), including its causes and treatments, remain major challenges to scientists and clinicians worldwide. The University of Alberta MS Centre seeks to advance our knowledge of MS, prompting us to seek ways to develop new knowledge, biomarkers and treatments to facilitate treatment and ultimately a cure for MS. Despite the COVID-19 pandemic the MS Centre has continued to flourish and now boasts thirty members comprised of scientists and clinicians. This is complemented by the very active MS Clinic, located in the Kaye Edmonton Clinic that cares for over 4000 persons with MS in northern Alberta.

We are extremely excited to present an outstanding lineup of young and dynamic speakers today who represent the future of MS research. They are from within the University of Alberta as well as from across the country. Indeed, we are also excited to host our keynote speaker Professor Lawrence Steinman who is a recognized leader in the field of MS globally. We continue to pursue our mission of offering a forum of interdisciplinary discussions that promote cross collaboration between experts in multiple sclerosis, their trainees and people affected by multiple sclerosis. Indeed, the MS Centre's mandate to advanced research, education, and innovative clinical care stands fast today despite the challenges of COVID-19 and changes within the University of Alberta. As always, we strongly encourage trainees and attendees at the symposium to network with our speakers. We also would like to thank everyone who has helped organize the research symposium including Tess Gleason for her dedication to organizing the symposium as well as our sponsors for their support of this event. Enjoy the day!

Bradley Kerr PhD & Christopher Power MD

Co directors, University of Alberta MS Centre

Co-Directors

Dr. Power is a Professor at the University of Alberta who studies the mechanisms of neuroinflammatory diseases and their treatments. In addition to directing his laboratory, the Brain Power Lab, he is also an attending physician in the University of Alberta HIV and MS Clinics and is the founding Director of the University of Alberta MS Centre.

Dr. Bradley Kerr received his BSc in Psychology from McGill University. He then went on to obtain a Ph.D. in Neuroscience from the University of London-King's College in the UK. Dr. Kerr joined the Department of Anesthesiology and Pain Medicine at the University of Alberta in 2007 and is also an adjunct professor in the Department of Pharmacology and Psychiatry. The focus of research in his lab is aimed at addressing the mechanisms of chronic pain after injury or disease with a major focus on chronic pain associated with Multiple Sclerosis.

Speaker 9:00 AM

Dr. Anastassia Voronova

University of Alberta

Title of presentation: Promoting brain regeneration and re-myelination from endogenous neural stem cells

Dr. Anastassia Voronova obtained her Ph.D. degree from the University of Ottawa, where she studied the regulation of embryonic stem cells. During her postdoctoral fellowship at the Hospital for Sick Children in the laboratory of Drs. Freda Miller and David Kaplan, she made seminal discoveries on how microenvironment controls neural stem cell biology. Dr. Voronova's independent research program at the University of Alberta focuses on engaging endogenous neural stem cells for brain regeneration in multiple sclerosis. She holds a Canada Research Chair in neural stem cell biology, and was awarded a Sloan Research Fellowship in Neuroscience in 2023 and an outstanding young investigator Jordi Folch-Pi award by the American Society for Neurochemistry in 2022.

Speaker 9:40 AM

Dr. Shannon Kolind

University of British Columbia

Title of presentation: Making Myelin Imaging Using MRI Accessible to Everyone

Dr. Kolind earned her PhD in Physics at the University of British Columbia (UBC) and a postdoctoral fellowship at the University of Oxford and King's College London, developing ways to measure myelin, the insulating layer that surrounds nerves in the brain and spinal cord, using magnetic resonance imaging (MRI). As an Associate Professor in Neurology at UBC, Dr. Kolind's lab is focused on developing a toolbox of tissue-specific imaging techniques and making them available to everyone, everywhere. Her multi-disciplinary team employs these multi-modal tools to achieving greater sensitivity and specificity in clinical research; particularly for clinical trials of new therapies.

Speaker 10:35 AM

Dr. Hedwich Kuipers

University of Calgary

Title of presentation: Microenvironmental control of and by inflammatory astrocytes

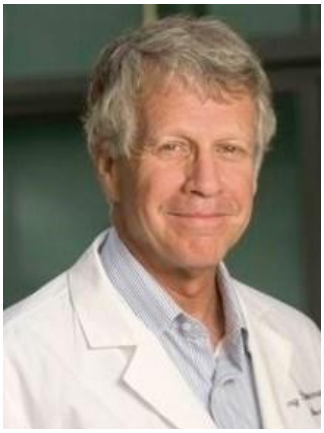
Dr. Kuipers received her MSc in Biopharmaceutical Sciences and her PhD in Immunology (cum laude) at Leiden University in the Netherlands. After this, she moved to Stanford University to do neuroimmunology research as a postdoctoral scholar in the lab of Dr. Lawrence Steinman and continued to study extracellular matrix immunology in the lab of Dr. Paul Bollyky as a research scientist. In April 2018 she joined the Departments of Clinical Neurosciences and Cell Biology & Anatomy of the University of Calgary as an Assistant Professor of Neuroimmunology. She is a member of both the Hotchkiss Brain Institute and the Snyder Institute for Chronic Diseases.

Speaker 11:15 AM

Dr. Lawrence Steinman

Stanford University

Title of Presentation: Deciphering the Specificity of Clonal Ig In the CSF to Explain the Pathogenesis of Multiple Sclerosis (live video)



Dr. Lawrence Steinman is a professor of Neurology and Neurological Sciences, Pediatrics, and Genetics. .

Dr. Steinman's research focuses on what provokes relapses and remission in multiple sclerosis (MS), the nature of the molecules that serve as a brake on the brain inflammation, and the quest for a tolerizing vaccine for autoimmune diseases like type 1 diabetes and neuromyelitis optica. He has developed two antigen specific therapies, using DNA vaccines, for MS and type 1 diabetes. He was senior author on the seminal 1992 Nature article that reported the key role of a particular integrin in brain inflammation. This research led to the development of the drug Tysabri, which is used to treat patients with MS and Crohn's disease.

Dr. Steinman received his BA from Dartmouth College and his MD from Harvard University. He was a post-doctoral fellow in chemical immunology fellow at the Weizmann Institute of Science in Israel. Dr. Steinman returned to Stanford University Hospital as a resident in pediatric and adult neurology and then joined the faculty at Stanford in 1980.

Speaker 2:00 PM

Dr. Jason Plemel

University of Alberta

Title of presentation: A diverse microglial response promotes remyelination

Dr. Jason Plemel is a Canada Research Chair in Glial Neuroimmunology and Assistant Professor in the Department of Medicine, Division of Neurology at the University of Alberta. His laboratory investigates microglia – our brain and spinal cord's primary immune cells – and the role they play during regeneration and injury to white matter in multiple sclerosis (MS). His lab is making strides to understand how these microglia are both critical for white matter regeneration, but also contributing to white matter injury. They are working to understand the mechanisms of white matter degeneration, as well as the complex immune response following white matter injury.

Speaker 2:40 PM

Dr. Anna Taylor

University of Alberta

Title of presentation: Pain and the emotional brain: what animal models can tell us about why MS hurts

Dr. Anna Taylor is a Canada Research Chair in Pain and Addiction, an assistant professor in the Department of Pharmacology and a member of the Neuroscience and Mental Health Institute at the University of Alberta. Dr. Taylor's research program engages a broad range of disciplines including pharmacology, microbiology, genetics, and animal behaviour to provide mechanistic insight into how affective circuitry contributes to pain and addiction.

Speaker 3:45 PM

Dr. Catherine Larochelle

Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM)

Title of presentation: Investigating metaflammation and inflammaging in MS

Dr. Larochelle obtained a MD-MSc degree (neurobiology) from Université Laval before completing her Neurology residency at Université de Montréal in 2009, and her PhD degree in neuroimmunology under the supervision of Dr Alexandre Prat in 2014. She then conducted a post-doctoral fellowship at the Johannes-Gutenberg University, Germany, under the supervision of Dr Frauke Zipp. She joined the CRCHUM in 2016 as a clinician-researcher in the Neurosciences department of Université de Montréal. Her research program focused on understanding and targeting immune-mediated neuroglial injury for neuroprotection in Multiple Sclerosis is supported by grants from the CIHR, FRQ-S and MS Society of Canada

Event Information

8:45 Welcome & Introduction, President Bill Flanagan

Bill Flanagan, President and Vice-Chancellor of the University of Alberta will open the symposium. Then the Co-Directors for the UofA MS Centre Drs. Bradley Kerr & Christopher Power, will introduce the day's events.

10:20 Break

12:15 Lunch

12:15 Trainee Posters

The MS Research trainees will be presenting their posters in the Atrium during the lunch hour. Come view them and ask questions.

1:30 Presentations by T. MacKeigan (UCalgary) and B. Hammond (UAlberta)

Two trainees who were chosen to give an oral presentation at the symposium.

3:20 Break

4:15 Open Question and Answer Session

Join in to our Question-and-Answer session in which members of the public and people affected by MS can interact with and have their questions addressed by our panel of MS experts.

17:00 Closing remarks

Trainee Abstracts

Trainee Abstract: Hajar Miranzadeh

Gasdermin B expression in glia contributes to cell death in progressive multiple sclerosis Authors: Hajar Miranzadeh[1], W.G. Branton[1], L. Schmidt[3], C. Power[1,2]

[1]Department of Medicine (Neurology)[1], University of Alberta
[2]Department of Medical Microbiology & Immunology, University of Alberta
[3]Laboratory Medicine & Pathology, University of Alberta

Background: Progressive MS (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 neu- ronal death. Previous studies implicated inflammasome activation and inflammatory regulated cell death, termed py- roptosis in myeloid cells. Gasdermin B (GSDMB) is a unique member of the gasdermin family that undergoes prote- olytic cleavage to cause pyroptosis. GSDMB expression in the CNS is unknown. We investigated GSDMB expres- sion in CNS and its contribution(s) to cell death in P-MS.

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

Results: We observed that GSDMB was highly induced in P-MS brains compared to nonMS brains based on RNASeq and RT-PCR analyses while immunodetection revealed GSDMB to be expressed in oligodendrocytes and astrocytes in P-MS brains. Transfection of the N-terminal of GSDMB resulted pyroptosis in transfected astrocytes and oligodendrocytes (MO3.13) in contrast to the transfected full-length or C-terminus of GSDMB.

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

Trainee Abstract: Madelene Faye S. Ho

Determining the Infiltrating Macrophage Contribution to Peripheral Neuropathic Pain

Supervisors: Dr. Bradley J. Kerr[1,4,5]*, Dr. Jason R. Plemel[1,2,3]*

Additional Authors: Sophia Khan[1], Tatiana Penina[1], Michelle Mathew[1], Alessandra Argandona[1], Gustavo Tenorio[4], Kelly Lee[1], Charbel Baaklini[1], Olivia La Caprara[5], Sameera Zia[1], Timothy Friedman[1], Aislinn Maguire[1], Brady Ham- mond[1], Dania Villareal Andrade[1]

Affiliations: [1]Neuroscience and Mental Health Institute, [2]Department of Medicine, Division of Neurology, [3]Depart- ment of Medical Microbiology & Immunology, [4]Anesthesiology and Pain Medicine, Faculty of Medicine and Dentis- try, [5]Department of Pharmacology, Faculty of Medicine and Dentistry, *contributed equally

Introduction: Neuropathic pain (NeP), an incurable chronic pain condition typically rated as “the worst pain possible” by patients. NeP can present in a multitude of conditions from the phantom-limb pain of peripheral nerve injury induced by amputations to systemic pain from autoimmune diseases like multiple sclerosis. NeP's well-characterized phenotype of prolonged hypersensitivity is initiated by macrophages of the nervous system. While the peripheral nervous system (PNS) is a critical contributor to establishment of long term chronic centralized pain, it is also important to understand as a potential target for therapy, as the PNS is easier to access, takes advantage of peripheral regenerative afferents, and therapy targets in the PNS do not need to cross a blood brain barrier such as in the CNS. Recent studies on peripheral nerve injury show the importance of infiltrating macrophages; it has been demonstrated that macrophage density in the DRG increase after nerve injury. However, the relative contributions of the infiltrating macrophage population are still poorly understood as approaches used to date fail to distinguish these infiltrating macrophages derived from circulating blood monocytes (monocyte-derived macrophages, MDMs) from resident macrophages.

Objectives: To investigate the role of MDMs under peripheral neuropathic pain, I aimed to characterize the dynamics of MDMs under NeP injury in the dorsal root ganglia (DRG, cluster of sensory neurons of the PNS) in a mouse model of NeP.

Design and Methods: Using novel transgenic mouse lines to fluorescently tag MDMs with tdTomato (tdT), we bet- ter define the localization, dynamics, and morphologies of DRG infiltrating macrophages in the spared nerve injury (SNI) model of NeP. We co-stain to investigate proliferation of MDMs. We treat the SNI mice with anti-CD49e, which prevents monocyte infiltration, to investigate infiltration-dependent nociception.

Main Results: tdT+ cell (MDMs) density in the DRG increases at 7 days post-injury (DPI) compared to naïve con- trols. Ki67+ proliferating cells increase in density at 7 DPI in the DRG, but these Ki67+ cells are not tdT+. We observe no sex differences in the Ki67+ tdT+ cells in 7DPI compared to naïve controls. We observe that mice injected with anti-CD49e acutely after SNI prevented allodynia during treatment timespan. We observe that MDMs adopt a “satellite” morphology post-injury (“spooning”). Spooning macrophages are found adjacent to neuronal cell bodies in the DRG, suggesting MDMs adopt direct interactive roles alongside peripheral somatosensory neurons.

Conclusions: Using mouse lines and injury models as tools to study the roles of MDMs provides context for future targets for immune-based NeP therapies.

Trainee Abstract: Tatiana MacKeigan

Fluorescence Spectroscopy for the Detection of Misfolded Protein Pathology in a Chronic Mouse Model of MS

Tatiana P. MacKeigan, Megan L. Morgan, Peter K. Stys

Abstract

Multiple sclerosis (MS) is one of the most common causes of neurological disability in young adults, with limited treatment options for disease progression. MS pathobiology is poorly understood, with components of autoimmune inflammation and an underlying degeneration of myelin and axons. The cuprizone (CPZ) mouse model is commonly used to study de- and remyelination. Mice display progressive white matter degeneration despite initial myelin repair after toxin withdrawal. To test whether this delayed demyelination is due to a progressive proteopathy, mouse brain sections harvested 8 months after a demyelinating CPZ insult were stained with the amyloid probe Thioflavin-S (ThS), and the corpus callosum (CC) examined via advanced fluorescence spectroscopy. Post-CPZ mice displayed a subtle but significant difference in the spectral character of ThS in the CC, which was eliminated with formic acid, providing strong evidence for deposition of misfolded proteins facilitating chronic white matter degeneration. This “late post- CPZ” model could represent a new, realistic animal model of progressive MS, allowing development of novel thera- pies for this phase of the disease. Our data also raise the possibility that the underlying cytodegeneration in MS may be driven by toxic amyloid accumulation as in many other traditional neurodegenerative disorders.

Trainee Abstract: Sameera Zia

Microglia diversity during remyelination and aging

Sameera Zia[1], Charbel Baaklini[1], Kelly Lee[1], Madelene F.S Ho[1], Sarthak Sinha[2], Mena Burr[1], Abhisha Patel[1], Andre Faria[1], Brady Hammond[1], Aislinn Maguire[1], Olivia La Caprara[3], Timothy Friedman[1], Bradley J. Kerr[1,3], Anastassia Voronova[1,4], Jeff Biernaskie[2,5], Jason R. Plemel[1,6,7]

[1]Neuroscience and Mental Health Institute
[2]Department of Comparative Biology and Experimental Medicine
[3]Department of Anesthesiology and Pain Medicine
[4]Department of Medical Genetics
[5]Hotchkiss Brain Institute and Department of Clinical Neurosciences, University of Calgary
[6]Department of Medicine
[7]Department of Medical Microbiology & Immunology

Multiple sclerosis (MS) is a neurodegenerative condition characterized by demyelinating lesions resulting from loss of myelin. Lost myelin may be regenerated through remyelination, which occurs spontaneously in people living with MS but is highly variable and loses efficacy with age. Remyelination is associated with reduced disability; however, promoting remyelination in people with MS remains an under-researched therapeutic strategy. Remyelination is promoted by microglia, the resident immune cells of the central nervous system that form unique subpopulations dependent on their microenvironments. *We hypothesized that microglia form distinct subpopulations specific to re- myelination that are altered with age.*
Methods: We isolated microglia throughout remyelination in the lysolecithin mouse model and used single-cell RNA sequencing to determine the subpopulations present. All results were validated using flow cytometry and RNAscope. **Results:** We found diverse remyelination-associated microglia (ReAM) characterized by the expression of *Igf1, Irf7, or Plp1 genes. Igf1 and Irf7-ReAM were specific to early remyelination while Plp1-ReAM emerged at the later stages. Igf1-ReAM demonstrated the most metabolic activity, Irf7-ReAM were modelled to be a transition state and Plp1- ReAM contain myelin transcripts likely indicative of myelin pruning. Igf1-ReAM were delayed with age while the Plp1- ReAM were nonexistent suggesting a delay in the microglial transitions with age.*

Trainee Abstract: Sharmistha P. Panda

Age-associated microglia/macrophage response inhibits remyelination

Sharmistha P. Panda1, Charbel S. Baaklini1, Kelly Lee1, Sameera Zia1, Brady P.Hammond1,Madelene F. S. Ho1, Olivia La Caprara2, Bradley J. Kerr2 , Jason R Plemel1

1- Department of Medicine, Neuroscience and Mental Health Institute,
University of Alberta, Edmonton, Alberta, Canada
2- Department of Anaesthesiology and Pain Medicine, University of Alberta,
Edmonton, Alberta, Canada.

Multiple sclerosis (MS) is a chronic inflammatory disease characterized by central nervous system (CNS) lesions, resulting in axonal loss as well as 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 an increase in 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 will use the LPC (lysophosphatidylcholine) model of demyelination and we plan to first examine 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 Ai9 mice. We find that age is associated with a delayed accumulation of microglia, but not monocyte derived macrophages, at 7 days post injection (DPI). Thus, age delays microglia response in the lesion area, potentially contributing to age-dependent remyelination decline. In the future we will then isolate and identify ROS-producing microglia/macrophage populations using a new single-cell RNA sequencing approach called ToxSeq and understand their interaction with OPCs during remyelination. We will also test Setanaxib, an inhibitor of the ROS-producing enzyme NOX inhibitors, to determine whether it reduces ROS production and improves remyelination in middle-aged mice. Thus, this study will examine the role of aged microglia/macrophages during remyelination and provide new directions to treat remyelination in aging people with MS.

Trainee Abstract: Sana Bibi

Role of MS-associated fractalkine variant in mouse brain development

Sana Bibi1, Monique M.A. de Almeida1, Yana Kibalnyk1, Adrianne Watson1 Kara Goodkey1, Astrid E. Cardona2 and Anastassia Voronova1

1 Department of Medical Genetics, University of Alberta, Canada 2 Department of Biology, University of Texas at San Antonio, USA

Multiple sclerosis (MS) is a demyelinating disorder where the immune system attacks myelin and myelin-producing cells oligodendrocytes in the central nervous system (CNS). The genetic basis affects both susceptibility and severity of MS. MS severity risk genes cluster into CNS development category; however, the role of MS severity risk variants in development is not known. Mutations in CX3CR1, a receptor for chemokine fractalkine (FKN), are associated with MS severity. Our lab showed FKN-CX3CR1 signalling regulates developmental oligodendrocyte formation. However, whether MS- associated CX3CR1 variants affect brain development is unknown.

To address this knowledge gap, we analyzed brains from developing and adult mice that express MS-associated human pathogenic CX3CR1I249/M280 variant (hM280). In comparison to WT, hM280 brains display i) developmental delay in oligodendrocyte formation and myelination; ii) increased activation of microglia and aberrant levels of cytokines in the developing brain; and iv) anxiety in adulthood.
In summary, mice expressing hM280 variant have delayed myelination and aberrant immune environment during CNS development. These transient developmental defects may lead to aberrant brain function in adulthood. Our results suggest that individuals with MS-associated variants may display aberrant brain development, which may be important for early detection and contribute to mechanisms of neurodegeneration later in life.

Trainee Abstract: Niall M. Pollock

Concurrent gasdermin D activation in oligodendrocytes and microglia drives inflammatory demyelination in progressive multiple sclerosis.

Niall M. Pollock[1], Jason P. Fernandes[2], Eman Moussa[3], Brittyne Hlavay[1], William G. Branton[1], Melinda Wuest[3], Nazanin Mohammadzadeh[2], Laura Schmitt[5], Jason R. Plemel[1], Olivier Julien[3], Frank Wuest[4], Christopher Power[1,2]

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3 **Department of Biochemistry,**
4 **Department of Oncology,**
5 **Department of Laboratory Medicine & Pathology, University of Alberta, Edmonton AB**

Central nervous system inflammatory demyelination and axonal injury are hallmarks of progressive multiple sclerosis (P-MS). Gasdermins are proteins involved in programmed inflammatory cell death, the best studied of which is gasdermin D (GSDMD). Proteolytic cleavage of GSDMD in oligodendrocytes and microglia causes inflammatory cell hyperactivation and death. White matter from persons with P-MS showed significantly increased expression of GSDMD, NINJ1, IL-1β, and -18 with- in chronic active demyelinating lesions. Using the cuprizone (CPZ) model of P-MS in mice, the effects of knockout of Gsdmd were explored. Oligodendrocytes and microglia displayed increased Gsdmd immunoreactivity in the central corpus callosum (CCC) of CPZ-exposed $Gsdmd^{+/+}$ mice, associated with significantly greater demyelination and reduced oligodendrocyte precur- sor cell proliferation compared to CPZ-exposed $Gsdmd^{-/-}$ animals. Electron microscopy showed the CCC of CPZ-exposed $Gsdmd^{+/+}$ mice disclosed significantly increased G-ratios, accompanied by reduced axonal densities and total myelinated axons. Proteo- mic analyses revealed increased brain complement C1q proteins and hexokinases in CPZ-exposed Gsdmd-/- animals. [^{18}F]FDG PET imaging showed increased glucose metabolism in the hippocampus and whole brain with preserved neurobehavioral per- formance in $Gsdmd^{-/-}$ animals after CPZ exposure. Convergent GSDMD activation in both microglia and oligodendrocytes con- tribute to inflammatory demyelination and neuroaxonal injury, offering mechanistic insights into neuroinflammation in P-MS.

Trainee Abstract: Nicole Dittmann

Ligands secreted by striatal neurons promote oligodendrocyte regeneration by endogenous neural stem cells

Nicole Dittmann1,2, Pouria Torabi1,2, Adrianne Watson1, Scott Yuzwa3 and Anastassia Voronova1,2

1Department of Medical Genetics, 2Neurosciences and Mental Health Institute, Faculty of Medicine & Dentistry, University of Alberta, 3Department of Laboratory Medicine & Pathobiology, University of Toronto

Loss of oligodendrocytes and myelin is a well-recognized hallmark of multiple sclerosis (MS) pathogenesis. The replace- ment of lost oligodendrocytes is a proposed key therapeutic goal, which can be achieved by engaging resident adult sub- ventricular zone (SVZ) neural stem and precursor cells (NPCs), the largest NPC pool in the adult brain. Adjacent to the SVZ is the striatum, a region rich in GABAergic neurons. While striatal neurons are key regulators of motor behaviour, and increased motor behaviour (exercise) is well known to stimulate NPCs and brain regeneration, the role of cell-cell communication between striatal neurons and SVZ NPCs is not known.

Here, we show media conditioned by primary murine striatal neurons instruct SVZ NPCs to differentiate into oligodendrocytes. Computational modelling predicted over 40 striatal neuron ligands that could engage receptors expressed in SVZ precursors and modulate their cell fates. From these molecules, we demonstrate Ttr (transthyretin) increases oligodendrocyte genesis from SVZ NPCs *in vitro* and *in vivo*.

Our studies show striatal neurons secrete molecules, including Ttr, that instruct NPCs to form oligodendrocytes. Future work will address the mechanism of TTR-induced oligodendrocyte regeneration in MS mouse models.

Trainee Abstract: Davis Juell

Postural loss caused by EAE in a skilled string-pulling task in mice

Co-authors: Behroo Mizha Agha, Samsoon Inayat, Sirjeet Singh, Sven Meuth, Majid Mohajerani, Ian Q. Whishaw

Abstract: Multiple sclerosis (MS) is a debilitating disease that is compounded by early life onset, preferential effect on females and absence of effect treatment. Treatment for MS is examined using a mouse with Experimental Auto- immune Encaphalomyelitis (EAE) that displays motor symptoms in the limbs, especially the hind limbs. The purpose of the present study was to examine potential EAE symptoms in the forelimbs using the string-pulling task in which a mouse pulls in a string to obtain food using hand over hand movements. 14 C56BL/6J and 24 Thy1-GCaMP6 female mice between 8 and 14 months of age will be injected with the MOG35-55/CFA emulsion and with pertussis toxin solution to induce EAE. Using machine learning tools, I will identify the onset of pathology. These behavioral and ana- tomical methods will create an objective assessment of established stages of disease progression and investigate any associated anatomical differences by stage. With disease progression measured, treatment such as that our collaborators at the Meuth lab are investigating can be implemented at an early disease stage at which neural changes are still minimal.

Trainee Abstract: Andrej Roczkowsky

Peroxisome Injury in Multiple Sclerosis: Protective Effects of 4-Phenylbutyrate

Andrej Roczkowsky, Matthew A.L. Doan, Brittyne Hlavay, Manmeet K. Mamik, William G. Branton, Brienne A. McKenzie, Laura Schmitt, Gary Eitzen, Francesca Di Cara, Leina B. Saito, Melinda Wuest, Frank Wuest, Richard Rachubinski, Christopher Power

Multiple sclerosis (MS) is a progressive and inflammatory demyelinating disease of the central nervous system (CNS). Peroxisomes perform critical functions in the CNS that contribute to homeostasis. We investigated peroxisome injury and mitigating effects of peroxisome-restorative therapy on inflammatory demyelination in models of MS. Human autopsied CNS tissues, human cell cultures, and cuprizone-mediated demyelination mice were examined by RT-PCR, Western blotting, and immuno- labeling. The therapeutic peroxisome proliferator, 4-phenylbutyrate (4-PBA) was investigated in vitro and in vivo. White matter from MS patients showed reduced peroxisomal transcript and protein levels, including PMP70, compared with non-MS controls. TNF-αexposed microglia displayed reduced immunolabeling of peroxisomal proteins, PMP70 and PEX11β, which was prevented with 4-PBA. Hindbrains from cuprizone exposed mice showed reduced Abcd1, Cat, and Pex5l transcript levels, with concurrent increased Nlrp3 and Il1b transcript levels, which was abrogated by 4-PBA. In the central corpus callosum, Iba-1 and peroxisomal thiolase immunostaining after cuprizone exposure was increased by 4-PBA. 4-PBA also prevented a decrease in myelin basic protein and neurofilament heavy chain and protected against cuprizone-induced neurobehavioral deficits. Pe- roxisome injury in CNS-associated macrophages contributed to neuroinflammation and demyelination that was prevented by 4- PBA treatment. A peroxisome-targeted therapy might be valuable for treating inflammatory demyelination and neurodegeneration in MS.

Trainee Abstract: Brady P. Hammond

Single-cell microglial transcriptomics during demyelination defines a microglial state required for lytic car- cass clearance

Sameera Zia[1†], Brady P. Hammond[1†], Martin Zirngibl[1†], Anastasia Sizov[1], Charbel S. Baaklini[1], Sharmistha P. Panda [1], Madelene F. S. Ho[1], Kelly V. Lee[1], Apurba Mainali[1], Mena K. Burr[1], Sioned Williams[1], Andrew V. Caprariello[2], Christopher Power[1,3,4], Thomas Simmen[5], Bradley J. Kerr [1,6] and Jason R. Plemel[1,3,4]

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6 Department of Anesthesiology & Pain Medicine, University of Alberta, Edmonton, Canada

Microglia regulate the response to injury and disease in the brain. In white matter diseases microglia may cause demyelination, but how they do so remains unknown. To understand how microglia respond during demyelination, we fed mice cuprizone—a potent demyelinating agent—and assessed the dynamics of genetically fate-mapped microglia. First, we transcriptionally profiled microglia to track their dynamics. Second, we ablated microglia and used acridine orange to assess microglial removal of dying cells. Lastly, we treated serum-free microglial cultures to model aspects of cuprizone-induced demyelination and assessed the responses. The cuprizone diet generated a robust microglial response. Single-cell RNA sequencing revealed the presence of cuprizone- associated microglia (CAM). These CAM expressed a transcriptomic signature indicative of cytokine regulation and reactive oxygen species production with altered lysosomal and metabolic changes consistent with ongoing phagocy- tosis. Using acridine orange to monitor apoptotic and lytic cell death after microglial ablation, we found that micro- glia preferentially phagocytose lytic carcasses. In culture, microglia exposed to lytic carcasses partially recapitulated the CAM state, suggesting that phagocytosis contributes to this distinct microglial state during cuprizone demye- lination. Together, CAM transcriptionally resemble microglia in other neurodegenerative conditions with phagocyto- sis as a potential link between them these different conditions.

Trainee Abstract: Charbel S. Baaklini

Microglia regulate OPC recruitment and differentiation during remyelination

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Multiple sclerosis is an inflammatory disease characterized by the loss of myelin. Several therapies are available for people with MS that reduce disability. Yet, no treatment is available to regenerate lost myelin, a process known as remyelination. Remyelination is associated with lower disability in people with MS, highlighting the potential for remyelination-promoting therapies. Remyelination requires microglia and monocyte-derived macrophages (MDMs), but the distinction between how these cells regulate remyelination is still unknown. We hypothesize that microglia-specific ablation in an experimental model of MS will result in impaired remyelination. We induced focal demyelination by injecting the toxin LPC into the spinal cord of mice. Microglia were ablated by injecting diphtheria toxin into mice that expressed a cre-recombinase inducible diphtheria toxin receptor due to the inducible cre-recombinase mouse line $CX3CR1^{CreER}$. We collected tissue at 4, 7, 14, and 21 days post-lesion induction (DPL) and quantified OPC proliferation (PDGFRa, Olig2, Ki67), differentiation (CC1, Olig2) and death (CC1, CC3). We found that at 4DPL, OPC proliferation was impaired in the absence of microglia. By 14DPL, the absence of microglia was associated with reduced oligodendrocyte accumulation, with no change in oligodendrocyte death at the earlier time-point 7DPL, suggesting that microglia promote OPC differentiation. We also found that microglia ablation impaired remyelination using electron microscopy. Taken together, microglia regulate distinct stages of remyelination. By understanding how microglia regulate remyelination, we hope to find new ways to boost the microglial response and improve remyelination.

Trainee Abstract: Aislinn D Maguire

Sex differences in the inflammatory response of mouse dorsal root ganglia neurons: implications for pain in Multiple Sclerosis

Aislinn D Maguire, Timothy N Friedman, Fajr Haq, Gustavo Tenorio, Karen Buro, Bradley J Kerr

Women are not only more likely to get Multiple Sclerosis than men but they are also more likely to experience neu- ropathic pain in the disease. Neuropathic pain in MS may originate in the peripheral nervous system at the dorsal root ganglia (DRG), which houses primary pain sensing neurons (nociceptors). These nociceptors become hyperexcitable in response to inflammation, leading to peripheral pain sensitization and eventually central sensitization, which main- tains pain long-term.

Using the MS mouse model experimental autoimmune encephalomyelitis (EAE) as well as TNFα-stimulated primary mouse DRG neuron cultures in vitro, we sought to characterize peripheral sex differences which may under- lie disparities in MS pain. We found sex differences in the inflammatory profile of the DRG, and in the signaling pathways activated by TNFα in nociceptors. Given that TNFα signaling has been shown to impact on mitochondrial function, this led us to investigate sex differences in mitochondria. Our results demonstrate that male sensory neurons are more sensitive to mitochondrial stress than females, possibly making them more prone to neuronal injury. Under- standing these sex differences is an important first step toward our long-term goal of developing sex-specific treat- ments to halt pain development in the periphery before central sensitization is established.

Trainee Abstract: Dania Villarreal

Neurite outgrowth from sensory neurons in the context of cellular stress

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Chronic pain in diseases like **multiple sclerosis (MS)** arise from overactive immune cells which promote neuronal injury and hyperexcitability in sensory neurons. The purpose of this study is to explore the role of the **Inositol Requiring Enzyme 1 (IRE1) pathway** of ER on the stress response on neurite outgrowth of sensory **dorsal root ganglion (DRG)** cells, to better un- derstand the mechanisms that promote neurite outgrowth in chronic pain.

IRE1 downstream effectors are thought to promote regeneration. I study the effects on outgrowth in the context of two different models. Running exercise promotes outgrowth robustly in female DRG cultures. Knockout of the IRE1 protein abolished out- growth of DRG cultures in response to running exercise. DRG cultures show increased outgrowth with exposure to macrophage conditioned media compared to control media which is diminished in IRE1 knockout mice.

Outgrowth results suggest that ER stress signaling is critical for the increased plasticity of sensory neurons in response to in- flammatory or natural stressors. In female mice, running promotes neurite outgrowth that is IRE1-dependent, suggesting sex differences. Future studies will address how increase in plasticity is related to pain and whether targeting ER stress pathways may be a viable option to treat chronic pain.

Trainee Abstract: Adrianne ES Watson

Fractalkine enhances oligodendrogenesis and remyelination from CNS precursor cells

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Multiple Sclerosis (MS) is an autoimmune and neurodegenerative disorder that leads to the damage or loss of myelin, an insulating layer that coats and protects nerve axons, and myelin-producing cells, oligodendrocytes. Current disease-modifying therapies are ineffective for progressive MS, which is characterized by worsening of the disease with no improvement. Treatments for progressive MS could be achieved by stimulating the production of new oligodendrocytes from resident oligodendrocyte precursor cells (OPCs) scattered throughout adult brain tissue. These newly formed oligodendrocytes would in turn remyelinate the brain. Our lab has shown that chemokine fractalkine (FKN) stimulates oligodendrogenesis in the normal adult brain from neural stem cells (Watson et al. 2021 Stem Cell Rep). Here, we asked whether fractalkine enhances oligodendrogenesis and remyelination from parenchymal OPCs after a demyelinating injury.

Using cuprizone mouse model of demyelination, we show that infusion of fractalkine (CX3CL1) into the brain after demyelinating injury increases de novo oligodendrocyte formation and enhances remyelination in the corpus callosum (white matter tracts) and cortical grey matter (de Almeida and Watson et al. 2023 Stem Cell Rep). We further show this is achieved by increased OPC proliferation

in the cortical grey matter and by attenuation of microglia/macrophage activation both in corpus callosum and cortical grey matter. Finally, we show activated OPCs and microglia/macrophages express fractalkine receptor CX3CR1 in vivo, and that in OPC- microglia co-cultures fractalkine increases in vitro oligodendrocyte differentiation by modulating both OPC and microglia biology. Thus, our results demonstrate a novel, pro-regenerative role of fractalkine after demyelination.
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Trainee Abstract: Uche J Ohaezukosi

Investigating the effect of normobaric oxygen therapy in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS

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Multiple sclerosis (MS) is a chronic neurological disease involving demyelination and inflammation. MS has been associated with significant reductions in cerebral blood flow (CBF) and brain hypoxia[1]. This may hasten CNS dam- age and disease progression in MS[1].

Previously, oxygen therapy was shown to slow the formation of demyelinating lesions in the spinal cord of an in- flammation-induced autoimmunity MS model called the experimental autoimmune encephalomyelitis (EAE)[2]. It is important to understand the effects of hypoxia and possible treatments for MS.

Methods: EAE mice were induced using myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adju- vant and pertussis toxin (CFA-PTX). EAE mice were randomly split into an untreated and treated group. Mice were assessed for symptoms using the 15-point clinical scoring scale. Oxygen treatment (100% normobaric) was started at the onset of symptoms (~Day 14), for 6 hours a day for 5 days. We imaged after treatment for assessment of CBF and hypoxia in the cortex and hippocampus using MRI. Behavior was assessed with an open field for changes in lo- comotion. We found no difference in the EAE-treated and Untreated post-treatment. However, there was a significant difference between the EAE and control groups in the MRI/behavior measures. Treatment generally didn't decrease disease progression.

References

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Trainee Abstract: Ziyan Zhu,

MRI Separation of Myelin and Iron in MS Lesions: One Year Longitudinal Study

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Introduction: Magnetic susceptibility (χ) separation may distinguish between paramagnetic (χ+) iron-laden microglia and dia- magnetic (χ-) myelin in MS lesions. We followed iron and myelin changes over 1 year in relapsing-remitting MS (RRMS) le- sions using χ separation.

Methods: 19 subjects with RRMS underwent two MRI scans with 14 months interval. Transverse relaxation (T2, T2*) and quantitative susceptibility mapping (QSM) enabled χ separation. Dominant iron and myelin lesion susceptibility changes were classified in relation to more standard QSM and T2* measures.

Results: 171 lesions were found in total. χ separation demonstrated clearer iron and myelin distributions in lesions, while QSM only provided the net sum. Regarding dominant myelin changes: 39 lesions (23%) had dominant χ- decrease over time repre- senting demyelination while 34 lesions (20%) had dominant χ- increase reflecting remyelination. Iron changes dominated the susceptibility in the remaining lesions. For 51 lesions (30%), with increased T2* and decreased QSM, most had χ+ reduction corresponding to iron loss. For the remaining 47 lesions (27%) with decreased T2* and increased QSM, most exhibited a χ+ increase, corresponding to iron gain.

Conclusion: χ separation provides anintuitive and quantitative assessment of simultaneous changes in iron and myelin enabling invivo monitoring of MS lesions.

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