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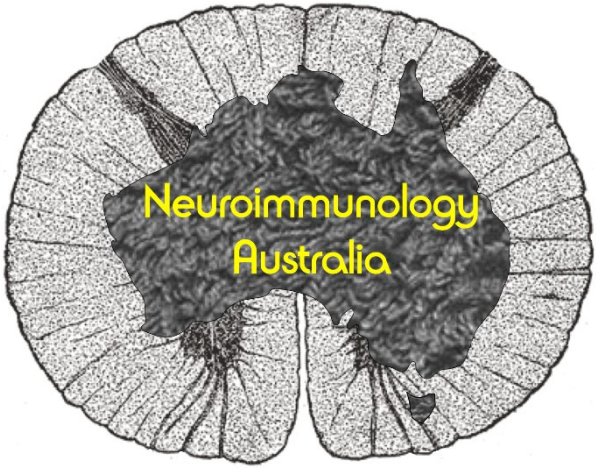

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Q&A on using artificial intelligence in academia (Chair: Fabienne Brilot)

POSTER abstracts

Live MOG-IgG cell-based assay: national accreditation on high-dimensional spectral flow cytometry

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Background: Seropositivity for MOG antibody (MOG-IgG) is paramount for MOGAD diagnosis. Flow cytometry live cell-based assays (CBA) are far superior to fixed CBA and a gold standard, albeit reputed as too bespoke for diagnostic accreditation. Herein, we investigated novel spectral flow cytometry, rarely used in diagnostics, and compared it to conventional technology in both research and diagnostic laboratories.
Methods: Human sera (n=24) were used to detect MOG-IgG by live CBA on three conventional cytometers (Fortessa, LSRII, Gallios), and two spectral cytometers (Aurora, ID7000). Fortessa, LSRII, and Aurora were research laboratory-based, whereas Gallios and ID7000 were diagnostic laboratory-based. ΔMFI was measured, serostatuses were determined, and inter- and intra-assay imprecisions were determined (CV%).
Results: MOG-IgG results are currently reported for clinical purposes using Fortessa, whereas accredited tests will be performed on ID7000. When compared, the Fortessa ΔMFI detection range was decreased by 5-fold and statistically significant vs the ID7000 range ($p=0.04$). However, both ranges were significantly correlated ($p<0.0001$; $R2=0.99$). Similarly, the ΔMFI detection ranges were also different between Fortessa and Aurora ($p=0.0001$). Comparison between spectral ID7000 and Aurora, showed that the difference in range was due to technology, and not due to laboratories. Intra- and inter-assay imprecisions were not different between the five cytometers. ID7000 and Fortessa had the lowest variation, with 4.6% and 6.8% intra-CV, respectively, and 15.7% inter-CV for both cytometers. FACS ratio, currently reported as a MOG-IgG titre proxy, and ΔMFI values were strongly correlated for all cytometers ($p<0.001$; $R2 \leq 0.99$). Results were

similar after analysis by either research FlowJo or diagnostic Kaluza softwares ($p<0.0001$, $R2=0.98$). Serostatuses were highly concordant ($\kappa=1$). Conclusions: Our results demonstrate that flow live CBA can be validated and accredited in diagnostic laboratories with high reproducibility and repeatability. In particular, improvement of these measures on the ID7000 strongly supports the use of spectral cytometry for diagnostic purposes.

Laboratory biomarkers for the prediction of relapse in myelin oligodendrocyte glycoprotein antibody-associated disease: a systematic review and meta-analysis

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Background: MOG antibody-associated disease (MOGAD), an inflammatory demyelinating pathology, is primarily diagnosed by detecting antibodies targeting oligodendrocyte-expressed MOG (MOG-IgG) in individuals with a typical clinical phenotype, such as acute disseminated encephalomyelitis (ADEM), optic neuritis (ON), or transverse myelitis (TM). Disease course is monophasic or relapsing. Laboratory biomarkers may improve disease course and activity prediction, thus, ensuring early and appropriate initiation of immunosuppression in individuals at risk of relapse-associated disability accrual, while minimising unnecessary immunosuppression exposure in monophasic individuals. Given the rarity of its incidence, MOGAD research has been challenged by limited sample sizes.

Methods: We performed a systematic review with meta-analysis and quantitatively assessed n=1710 individuals with MOGAD.

Results: Relapsing course was associated with persistent seropositivity (OR 2.79 (95% CI 1.90–4.11), p<0.0001), lower risk of seroreversion to negative status (HR 0.21 (95% CI 0.16–0.28), p<0.0001), and delayed seroreversion compared to monophasic individuals (median 19.08 years versus 2.92 years, respectively, p<0.0001). The highest rate of seroreversion to negative status occurred within 12-months following disease onset. Additionally, serum MOG-IgG titre (odds of attack with titre: negative (OR 0.07 (0.03–0.19)), low positive (OR 1.00 (reference)), clear positive (OR 3.61 (2.59–5.03)), p<0.0001) and cerebrospinal fluid leukocytosis (OR 3.11 (1.65-5.85), p=0.0004) significantly discriminated attack from remission, thus, may be

valuable in settings of clinical uncertainty for individuals with known MOGAD diagnosis to discriminate whether symptoms likely represent MOGAD attack as opposed to an alternate pathology. Neither serum glial fibrillary acidic protein (GFAP) nor neurofilament light chain (NFL) were significantly associated with attack. Multiple novel biomarkers of disease course and activity were identified and assessed qualitatively.

Conclusions: Longitudinal MOG-IgG serostatus appears to be a biomarker of MOGAD relapsing course. Clinicians may adopt serial measurement of serum MOG-IgG at 3–6-month intervals in the 12-months following onset to assist risk stratification of relapse and guide therapeutic decisions.

Targeting C5a Inflammation in ALS: Ex Vivo Findings from PMX205 Phase Ib Trial

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Amyotrophic lateral sclerosis (ALS) is characterized by progressive motor neuron degeneration, with growing evidence suggesting that inflammation, particularly via complement activation, contributes to disease progression. C5a-C5aR1 signalling is the major inflammatory byproduct of complement activation. Preclinical studies have demonstrated that C5aR1 blockade with PMX205, a selective C5a receptor 1 (C5aR1) antagonist, can mitigate neuroinflammation in ALS models. PMX205 is under investigation in Phase I human trials in ALS patients. In this study, we evaluated the effects of PMX205 in ALS patients by examining cytokine and immune response markers in blood samples.

Blood samples from eight ALS patients were collected before, and 2 hours after, PMX205 administration. Whole blood was then stimulated with vehicle (PBS) or C5a (10nM) for 24 hours. Plasma cytokine levels were measured using the Cytokine Bead Array Human Inflammation Panel (13 analytes). For a more comprehensive analysis, Somalogic proteomic technology was employed to assess 1,500 inflammatory and immune-related markers.

C5a stimulation at 10 nM significantly upregulated IL-1β, TNFα, IL-6, IL-8, and IL-10 in ALS patient blood samples. Paired blood samples taken from patients after PMX205 administration showed significantly reduced levels of these cytokine. Extended proteomic analysis revealed additional changes driven by C5a, with numerous cytokine and chemokine proteins showing significant upregulation, and modulation after PMX205 treatment. Comparing pre- and post-drug treatment levels highlighted a consistent reduction in key cytokines and chemokines, with the most notable changes in CCL and CXCL family members, and cytokines IL-6. These results underscore a robust pharmacodynamic effect of low-dose subcutaneously administered PMX205 in modulating C5a-driven inflammatory responses in ALS patients.

These findings provide critical pharmacodynamic support for further clinical evaluation of longer-term dosing of PMX205 in larger ALS cohorts. Future analyses using more detailed proteomics could provide deeper insights into the broader immune modulation effects of PMX205.

PAC1 Deficiency Exacerbates ER Stress and Impairs Myelin Regeneration in a Mouse Model of Multiple Sclerosis

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Multiple Sclerosis (MS) is a progressive central nervous system (CNS) disorder characterised by demyelination and neurodegeneration, often accompanied by motor and cognitive deficits. A core pathological mechanism in MS is endoplasmic reticulum (ER) stress, regulated by the Unfolded Protein Response (UPR). The PAC1 receptor, expressed abundantly in oligodendrocytes and neurons, has emerged as a potential therapeutic target due to its role in modulating ER stress.

This study investigates how PAC1 deficiency affects myelination, neuronal health, and ER stress during cuprizone-induced demyelination and spontaneous remyelination in mice.

Quantitative PCR revealed significant downregulation of key myelin markers (MOG, MBP, Olig2) in the motor cortex and white matter of PAC1-/- mice compared to PAC1+/+ controls ($p<0.0001$). Immunofluorescence analysis showed a reduction in mature oligodendrocytes (ASPA+ cells; $p<0.01$), and Luxol Fast Blue staining confirmed decreased myelin density in the corpus callosum of PAC1-/- brains ($p<0.05$). During demyelination, pIRE1—a key UPR marker—was significantly downregulated in PAC1-/- motor cortex ($p<0.05$), suggesting an impaired ability to manage cellular stress, which may contribute to the observed myelin loss.

This oligodendrocyte reduction persisted at both 1 and 3 weeks post-remyelination in the corpus callosum of knockouts, suggesting impaired myelin recovery in the absence of PAC1, but was reversed upon injection of PAC1 agonist Maxadilan ($p<0.05$). At 1-week post-remyelination, pIRE1 levels were elevated in PAC1-/- motor cortex, indicating prolonged ER stress may be limiting myelin repair. Behavioural testing (Open Field, Rotarod) showed that Maxadilan ameliorated cuprizone-induced motor coordination deficits compared to saline controls in PAC1+/+ mice ($p<0.05$).

These results demonstrate that PAC1 loss impairs both myelin maintenance and recovery, likely through dysregulated ER stress responses. Pharmacological activation of PAC1 can partially rescue these deficits, highlighting the PACAP/PAC1 axis as a promising therapeutic pathway for enhancing myelin repair and functional recovery in MS.

Spatial Mapping of Pro-inflammatory CD4+ T Cell Subsets and Migratory Cues in the Inflamed CNS

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Proinflammatory CD4+ T cells contribute to multiple sclerosis pathogenesis; specifically blocking their migration to the CNS is an attractive target for MS therapies. However, molecular mechanisms of migration to the CNS utilised by proinflammatory CD4+ T cells remain to be fully determined. To provide new insights, we used Xenium in-situ transcriptomics in the EAE model revealing spatial enrichment of migratory cues and receptors expressed by infiltrating T cells in the inflamed CNS. At peak EAE, a coronal section of brain and transverse sections of cervical, thoracic, and lumbar spinal cord were analysed using XeniumPrime5k, probing for >5000 genes with subcellular spatial resolution. Data were clustered and visualised in XeniumExplorer, and spatially enriched migratory signals were determined via Voyager, and upregulated communication pathways between infiltrating CD4+ T cells and other cell types in the CNS via CellChat. These revealed spatial enrichment of chemoattractant ligands and receptors associated with CD4+ T cells and other cells in the CNS. Notably, Cxcr6 ligand Cxcl16 is enriched in dendritic cells, macrophages, and microglia, and CXCR6-CXCL16 communication was highly upregulated. Cxcr6-expressing CD4+ T cells were visualised making close contacts with these cells. Additionally, Ccr2 ligand Ccl2, expressed by astrocytes and microglia, also demonstrates spatial localisation and upregulated pathway communication. We functionally assessed CXCL16 and CCL2 contribution to pro-inflammatory CD4+ T cell recruitment to the CNS in EAE. Blocking antibodies against CXCL16 and CCL2 in EAE mice substantially reduced disease severity and proinflammatory CD4+ T cell accumulation in the CNS, when given in combination. These data support a combinatorial role for CXCL16 and CCL2 in recruiting pro-inflammatory CD4+ T cells to the CNS during autoimmune inflammation. These spatial analyses are ongoing to reveal other migratory pathways driving proinflammatory CD4+ T cell infiltration to the inflamed CNS, which can be potentially harnessed as novel therapeutic targets in MS.

Investigating neutrophil-driven inflammatory T cells in CNS autoimmunity

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Inflammatory T cells are key mediators of central nervous system (CNS) inflammation in multiple sclerosis (MS) and are established drivers of autoimmunity in its experimental autoimmune encephalomyelitis (EAE) model. While the intrinsic factors promoting their generation are increasingly well-characterized, the extrinsic cell types that shape their pathogenic differentiation in vivo remain poorly defined. Recent studies have associated neutrophils and their secreted mediators with MS severity, and prior work in the EAE model has demonstrated that neutrophils are essential for disease induction. However, their specific roles in driving inflammatory T cell responses during autoimmunity remain unclear.

We have identified a novel role for neutrophils in promoting inflammatory T cell differentiation in the EAE model. Depletion of neutrophils during the preclinical phase of EAE significantly reduced the priming of inflammatory T cells in the spleen. Using the phenotypic markers (CD49d and CXCR2), we characterized immature and mature neutrophil subsets and isolated them via FACS for co-culture with CD4+ T cells. Strikingly, immature neutrophils selectively promoted the generation and proliferation of inflammatory T cells in vitro. This neutrophil-driven inflammatory T cell response was dependent on cell–cell contact and occurred independently of the previously described histone–TLR2 signaling axis in T cells. Ongoing work in the lab is focused on identifying the specific mediators expressed by immature neutrophils that are responsible for driving inflammatory T cell differentiation in this context.

Together, these findings uncover a previously unrecognized role for immature neutrophils as regulators of inflammatory T cell responses in CNS autoimmunity. Defining the mediators of this interaction will provide new insights into T cell–neutrophil crosstalk and may uncover novel therapeutic targets for MS.

Determining the Role of Adaptive Immunity in a Mouse Model for Aicardi-Goutières Syndrome

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Aicardi-Goutières syndrome (AGS) is a rare, genetically driven neuroinflammatory disorder caused by mutations in nine genes. It is characterized by excessive production of interferon-alpha (IFN-α), a pro-inflammatory cytokine, within the central nervous system (CNS). AGS typically presents within the first few months of life as a progressive encephalopathy, leading to developmental regression, cognitive and motor impairments, and high infant mortality.

Recent findings from our laboratory reveal a pivotal role for the brain's microvasculature—particularly the endothelial cells of the blood-brain barrier (BBB)—in mediating IFN-α-induced neurotoxicity. This vascular dysfunction contributes to microangiopathy or cerebral vasculopathy and promotes the infiltration of adaptive immune cells, including B and T lymphocytes, into the brain parenchyma.

To investigate the role of adaptive immunity in AGS pathogenesis, we crossbred our GIFN mice with CNS-targeted overexpression of IFN-α with Rag-1 knockout mice, which lack functional B and T cells, to obtain GIFN x Rag-1 KO mice. We hypothesized that infiltration of adaptive immune cells accelerates disease progression in GIFN mice.

Mice were studied at early (8 weeks) and late (16 weeks) disease stages. We conducted comparative clinical, behavioural, pathological, and molecular analyses of brain tissue from GIFN x Rag-1 KO mice and control groups (WT, GIFN, and Rag-1 KO). Preliminary results indicate a reduction in brain pathology and inflammation in GIFN x Rag-1 KO mice, along with improved motor coordination compared to GIFN mice with intact adaptive immunity. These findings suggest a contributing role of B and T cells in AGS progression and point to adaptive immunity as a potential therapeutic target.

Investigating CNS white matter defects in a murine model of Aicardi-Goutières syndrome

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Aicardi-Goutières syndrome (AGS) is a rare but devastating genetically inherited leukodystrophy that primarily affects newborns and paediatric patients. AGS predominantly impacts the brain and immune system, causing progressive motor and cognitive decline, lesions in the central nervous system (particularly in the white matter), developmental delays, and elevated levels of interferon-alpha (IFN-α) in the cerebrospinal fluid. Although AGS is classified as a leukodystrophy, the nature of white matter damage (WMD) remains poorly understood, and the role of IFN-α in disease pathology is not yet fully clarified.

To address this gap, we harnessed brain tissue from transgenic (GIFN) mice with chronic overexpression of IFN-α, driven by a glial fibrillary acidic protein promoter, at two different age groups (2 and 7 months), to study WMD.

Histological and immunohistochemical assessments were conducted on brain tissue sections from age-matched GIFN mice and wild-type littermates using Luxol Fast Blue (LFB), Haematoxylin and Eosin (H&E), Alizarin Red Staining (ARS), stereological counts of OLIG2+ and ASPA+ cells, and immunofluorescence for IBA1 (microglial marker). Reflecting the clinical course of AGS, GIFN mice exhibited an age-dependent progression of WMD. LFB staining revealed demyelination (areas of discoloration) in the corpus callosum and cerebellum, which was further supported by dysregulation of several myelin-related genes (MBP, PLP1, OLIG2, SOX10, and MOG). H&E staining identified diffuse microangiopathy and signs of vascular leakage, accompanied by dysregulation of endothelial markers such as KLF4 and eNOS. ARS revealed sparse cerebellar and cortical calcifications with increasing severity in older GIFN mice, while immunofluorescent analysis indicated chronic neuroinflammation and reduced myelination.

These findings further validate GIFN mice as a preclinical model for investigating AGS pathophysiology and the mechanisms underlying leukodystrophy, demonstrating age-dependent WMD and vascular abnormalities. By highlighting IFN-α-associated neuroinflammation and WMD, this model advances our understanding of AGS pathogenesis and supports the development of potential therapeutic strategies.

Dual roles of the brain microvasculature: From protector to driver of pathology

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Aicardi-Goutières syndrome (AGS) is a rare but devastating neurogenetic disorder. Children with AGS experience neurodevelopmental delays, physical impairment, and early mortality. While symptoms can vary, one unifying feature is chronically elevated levels of interferon-alpha (IFN-α) in the brain. Despite this shared hallmark, underlying cellular mechanisms remain poorly understood and treatment options remain limited to alleviating peripheral but not central symptoms. Our lab has recently identified that the brain microvasculature is a key target of IFN-α and that vascular damage precedes neuropathology, shining a light on the brain microvasculature as a pathologic driver of AGS.

To investigate the pathogenic role of the brain microvasculature in AGS, this study utilises an established human induced pluripotent stem cell (iPSC) line and CRISPR/Cas9 genome editing to introduce AGS mutations into the iPSC. These iPSCs are then differentiated into the main cell types that make up the brain microvasculature – endothelial cells, pericytes, and vascular smooth muscle cells.

Our results show that endothelial cells carrying an AGS mutation have a spontaneous and exaggerated response to IFN-α, which is not observed in mutated pericytes and vascular smooth muscle cells. AGS-mutated endothelial cells also exhibit impaired ability to form blood vessels in a 3D organoid model.

Together, these results suggest that AGS mutations have cell-intrinsic deficits that are specific to endothelial cells. While this highlights the importance of studying the brain microvasculature in AGS, it can also provide insights into neurological diseases characterised by immune and vascular dysregulation.

A parasitic worm derived ATPase modulator as a potential multifunctional therapeutic for multiple sclerosis

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Background: Several therapeutic agents are currently available that modify the disease course to treat exacerbations of the autoimmune demyelinating disease, multiple sclerosis (MS). But importantly, these drugs do not stop the chronic neurodegeneration that almost invariably develops over time in people with MS. Recently, we have been investigating a novel molecule, helminth defence molecule 1 (FhHDM-1), produced by the parasitic worm Fasciola hepatica, as a potential treatment for MS.
Methods: A relapsing-remitting experimental autoimmune encephalo-myelitis (RR-EAE) model of MS, in which mice were treated with a single short course of PBS or FhHDM-1 after they had recovered from an initial severe attack of EAE (at least both hind legs paralysed), was used to assess long term immunological and histological outcomes (70 days post onset of treatment). Autoreactive T cell responses to myelin antigens, cytokine responses, and other cellular responses were measured, and CNS tissue was assessed histologically and by mass spectrometry.
Results: FhHDM-1 inhibited macrophage activity and blood-brain barrier breakdown, but had no effect on adaptive immune responses. CNS pathology was minimal in FhHDM-1-treated mice 70 days post treatment onset, despite the initial severe attack of EAE prior to treatment initiation. Data from mass spectrometry analyses strongly suggest that the unifying element in FhHDM-1's protective effects involves its ability to significantly modulate several types of ATPases, including upregulation of mitochondrial ATP synthase, decreased activation of vacuolar ATPase (leading to decreased lysosomal acidification and zymogen activation and decreased lysosomal dyshomeostasis in neurons) and upregulation of P-type ATPases within the CNS (leading to increased propagation of nerve impulses).
Conclusion: The ATPases modulated by FhHDM-1 regulate many functions that are dysregulated in MS and that can lead to chronic neurodegeneration in MS (e.g. altered mitochondrial function and ion homeostasis). FhHDM-1 therefore appears to have the potential to prevent not only immune-mediated relapses, but also changes that can lead to neurodegeneration.

Region-specific cyst localisation and widespread innate immune activation in the retina and brain in a mouse model of acute toxoplasmosis

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Toxoplasma gondii, a globally distributed neurotropic parasite, is a leading cause of posterior uveitis and encephalitis. We investigated (i) whether *T. gondii* preferentially localises in different regions of the retina and brain in a mouse model, and (ii) whether resident microglia are activated in a region-dependent manner during acute infection.

C57Bl/6J mice were intraperitoneally inoculated with *T. gondii* Pru-tdTomato tachyzoites (low-dose [5x103] n=5; high-dose [1x104] n=5) or PBS (n=5). In vivo multimodal fundus imaging was performed every 7 days to monitor clinical disease and retinal parasite invasion. Eyes and brains were collected at day 28 post-infection; retinal wholemounts and brain sections were then processed for immunostaining (using Tmem119 and MHC class II antibodies) and confocal microscopy. *T. gondii* cyst burden and microglia density, field area and MHC class II expression were quantified using FIJI.

Retinal lesions, perivascular cuffing and tdTomato+ *T. gondii* parasites were observed in infected mice from day 14 using in vivo fundus imaging. Examination of fixed tissues revealed that *T. gondii* cysts were exclusively localised in the ganglion cell layer (GCL) and inner plexiform layer (IPL) of the retina, whereas in the brain a predilection for cyst formation in the cortex occurred. Despite this tissue tropism, microglia activation occurred throughout the retina and brain, evidenced by MHC class II upregulation and reduced microglia field area in all examined CNS regions (retina: GCL, IPL, and outer plexiform layer; brain: cortex, olfactory bulbs, hippocampus, cerebellum) compared to controls ($p<0.05$). *T. gondii* infection was also associated with a large increase in infiltrating MHC class II+ cells in the retinal GCL, and upregulation of MHC class II on brain vasculature.

In conclusion, *T. gondii* preferentially forms cysts in the innermost layers of the retina and the cerebral cortex; however, microglia activation is not restricted to these regions during acute infection.

Defining the impact of lesion level on intraspinal inflammation and wound healing through multi-omics

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Over half of spinal cord injuries (SCIs) globally are high-level lesions, disrupting CNS-immune communication. Higher-level SCIs are therefore associated with immune impairment. This is clinically relevant as infections remain a leading cause of death post-SCI and are thought to worsen recovery. While high-level SCIs are known to reduce immune cells in lymphoid organs and the blood, how this impacts local inflammation and wound healing at the lesion site remains unclear. To address this, we used spatial and single-cell multi-omics to explore the interplay between lesion level, inflammation and scar formation. Comparing high (T2) and low (T9) contusive SCI, mice with T2 SCI had worse motor recovery, autonomic dysreflexia, and immune depression-like symptoms. We spatially mapped cells across naïve, T2 SCI and T9 SCI, revealing a scar-associated gene module enriched for ECM and collagen genes, elevated in T2 lesions. Ligand-receptor analysis further revealed enhanced macrophage-fibroblast crosstalk in T2 SCI. To better discern immune-related genes or pathways associated with improved SCI outcomes (rather than a generalised wounding response), we created an scRNASeq atlas of all $CD45^+$ cells in T9 SCI ± IVIG (an immune-modulatory intervention). Sub-clustering revealed 42 immune cell types, 38% of which changed in abundance with IVIG. We further identified genes that were highly expressed in T2 SCI but reduced by IVIG or vice versa. Overall, our multi-omics atlas of inflammation and fibrosis in acute SCI uncovered diverse immune states, their spatial patterns, and lesion-level differences in fibrotic scarring. We are now beginning to dissect the role of regulated inflammatory genes and pathways in SCI wound healing responses, so we can target these therapeutically to improve outcomes.

Characterisation of neuroinflammation in growth-restricted postnatal day 10 piglets

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Background: Fetal growth restriction (FGR), commonly caused by placental insufficiency, has a high risk for adverse long-term neurodevelopmental outcomes, including cerebral palsy. Recent studies suggest that neuroinflammation is one of the key contributors to impairment of grey and white matter development in the FGR brain. This study aims to characterise the inflammatory response and examine sex effects in the prefrontal cortex (PFC) and basal ganglia (BG) in postnatal day 10 (P10) piglets.

Methods: Newborn piglets with spontaneous fetal growth restriction (FGR; birth weight <10th percentile) and normally grown (NG) littermates were studied from postnatal day 1 (P1) to postnatal day 10 (P10) (FGR = 8; NG = 9). Immunohistochemistry was performed to assess neuroinflammatory response (active microglia, Iba-1 and reactive astrocytes, GFAP) and cell death (Caspase-3) in the PFC and BG (Caudate nucleus (CN), Putamen(P) and Globus Pallidus (GP).

Results: At P10, FGR piglets displayed a significant increase in microglial (PFC $P = 0.0004$; BG $P<0.0001$), astrocytic activation (PFC and BG $P< 0.0001$). We observed increased apoptosis, as assessed by Casp3 labelling, in all regions of the grey matter ($P< 0.0001$) and white matter (PFC: $P<0.0001$; CN and P: $P<0.05$) when compared to NG. Sex differences were not observed within phenotypes, with male and female FGR displaying comparable increases in each marker assessed relative to NG.

Conclusion: These findings indicate glial activation in the developing brain of FGR neonates, which may be associated with neuroinflammation. Ongoing glial activation was observed in all regions examined, along with increased apoptosis. No sex differences were observed for any markers or regions examined. These findings suggest that persistent neuroinflammation in FGR leads to glial activation, promotes cell death and loss of neuroprotective functions, and potentially contributes to grey and white matter impairment, warranting further investigation.

Poster Abstracts

Poster

Interleukin-6 Classical and Trans Signalling have Overlapping and Distinct Gene Expression Responses

Manuela Estphan*, Katia Reis*, Victor Caxala, Markus J Hofer
**: co-presenting*

Charles Perkins Centre and School of Life and Environmental Sciences, The University of Sydney.

Microglia are the resident immune cells of the central nervous system. These cells can adopt pro- and anti-inflammatory states, which promote and resolve neuroinflammation respectively. The inflammatory state of microglia is meticulously regulated by cytokines including interleukin-6 (IL-6). The availability of two IL-6 receptor forms – transmembrane and soluble - allows this cytokine to signal via two distinct mechanisms: classical and trans, respectively. Current literature postulates that both signalling mechanisms activate identical downstream signalling cascades including the STAT3, MAPK and AKT pathways. However, on a functional level, trans signalling is characterised as pro-inflammatory, while classical signalling is characterised as anti-inflammatory. The underlying differences that drive these distinct functional outcomes remain a gap in literature. In this study, the gene expression profiles of IL-6 classical and trans signalling were determined in primary murine microglia using single-cell sequencing. The results revealed both overlapping and distinct sets of differentially expressed genes between the two pathways. The key distinction appears to be the induction of interferon-stimulated genes in classical signalling, implicating the unique activation of STAT2 and IRF9 transcription factors in this pathway. Furthermore, this study suggests that both classical and trans signalling are pro-inflammatory in nature. Further experiments are currently underway to validate and expand upon these results. This study opens new avenues for exploring global IL-6 blockade as a therapeutic strategy for managing dysregulated neuroinflammation in various diseases, moving beyond a trans-specific focus.

Poster

Proteomic plasma screening in fetal growth restriction piglets reveals altered pathways

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Fetal growth restriction (FGR) impacts 5-10% of pregnancies. Although the causes are multifactorial, one of the most common causes of FGR is placental insufficiency. FGR is associated with increased risk of mortality and morbidity however, diagnosis of FGR does not indicate the level of risk for an individual infant. Identifying alterations to the plasma proteome is an important step towards identifying mechanisms and targets for treatments. Here we used an established spontaneously occurring piglet model of FGR and liquid-chromatography mass spectrometry (LCMS) to identify alterations in the plasma proteome when compared to normally grown (NG) piglets. Blood plasma samples from 15 FGR (birthweight <10th percentile and brain:liver weight ratio ≥1) and 14 NG piglets of mixed sex were collected <24 hours after birth. Plasma samples were prepared for LCMS and subsequently analysed using Zeno SWATH MS at UQCCR MS facility. Dysregulated proteins were identified and then investigated for pathway and network associations using Ingenuity Pathway Analysis (IPA). A total of 106 proteins were determined to be dysregulated in FGR compared to NG piglets. Of these, 62 were decreased and 44 were increased in abundance in FGR. IPA revealed associations with pathways and functions consistent with previous findings such as immune and inflammatory responses and cardiovascular and neurological diseases. Protein-protein interaction networks identified novel alterations associated with FGR including increases in Fetuin A predicted to impact AKT expression. This is the first study to our knowledge to screen the proteomic profile of FGR piglet plasma. Consistency with previous studies indicates the robustness of these results. The protein interaction networks demonstrated multiple interacting pathways involved in FGR, many of which are novel findings. These findings provide crucial direction for future studies by adding to our understanding of mechanisms involved in FGR and clinically as a foundation for therapeutic targets.

Poster

CD93: A New Complement-Linked Target for Immunomodulation and wound healing in SCI

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Background: CD93, also known as C1qRp, is a transmembrane protein historically implicated in endothelial cell function and angiogenesis. Emerging evidence now also supports a broader role for CD93 in immune regulation and ECM remodelling. Interestingly, CD93 has been associated with both protective and pathological responses, with this duality seemingly dependent on the disease context. We identified CD93 as being upregulated in traumatic spinal cord injury (SCI), a condition marked by chronic dysregulated inflammation and scarring, but its role in this pathology remains poorly understood.
Methods: We performed single-cell RNA sequencing (scRNA-seq) and immunofluorescent staining to map CD93 expression at various time points (up to 42 days) after contusive SCI in mice. We used cell marker genes and/or antibody approaches, respectively, to identify the various cell types expressing CD93.
Results and Conclusion: We found that CD93 is robustly expressed at the site of SCI in association with both blood vessels and myeloid cells, including subsets of neutrophils, monocytes and/or monocyte-derived macrophages, and microglia. These findings provide a basis to further explore a functional role for CD93 in relation to neuroinflammation and spinal cord wound healing after injury.

Poster

Mapping the unseen: Exploring Complement System Dynamics in Alzheimer's Neuroinflammation

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The complement system as part of the immune system plays an important role in regulating inflammation. It consists of a group of proteins that recognize, opsonize and eliminate pathogens. In addition, the complement system contributes to the development and homeostasis in the brain by contributing to synapse pruning and removal of dead cells.
Alzheimer's disease is a progressive neurodegenerative disorder that primarily affects memory and cognitive functions. It is characterized by the accumulation of beta-amyloid plaques and tau tangles, and loss of neurons. In recent years, there has been growing evidence suggesting that the complement system plays a role in the development and progression of Alzheimer's disease. Studies have shown that certain complement proteins, such as C3 and C4, are present in higher levels in the brains of individuals with Alzheimer's disease. These proteins can interact with beta-amyloid plaques and tau tangles, leading to chronic inflammation and promote damage to nerve cells. Furthermore, genetic studies have identified specific gene variants associated with both Alzheimer's disease and the complement system.
Here we investigated the expression and localisation of several complement proteins in different brain regions from patients with various stages of Alzheimer's disease. Our findings reveal differential expression of complement factor genes in different disease stages and brain regions that correlate with disease pathology.

Poster

Integrated Experimental & Mathematical Approaches to Modeling Neuroinflammation

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Neuroinflammation is driven by cellular interactions that are difficult to model with experimental or mathematical approaches alone. Microglia, the resident immune cells of the central nervous system, dynamically shift between functional states in response to different stimuli. Here we present an integrated framework that combines microscopy, cell quantification, and RNA sequencing with mathematical modeling to describe the cellular interactions underlying neuroinflammation. Our data reveals four distinct microglia subtypes whose behaviors and interactions with other neural cell types are incorporated into a system of ordinary differential equations. This approach allows us to move from population-level description of microglia to a subtype-specific description of their roles in inflammation. Using our mathematical model, we explore how microglia subpopulations differentially contribute to the propagation of the neuroimmune response, specifically through cytokine secretion, blood-brain barrier weakening, and T cell activation. We will also outline how our suite of primary experimental data can be used by others to inform future mathematical models.

Poster

The effects of the neurotropic parasite *Toxoplasma gondii* on microglia, astrocytes and neurons during experimental chronic infection in mice

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Toxoplasma gondii, a globally prevalent protozoan parasite, invades the central nervous system (CNS) and establishes chronic infection. The long-term consequences of *T. gondii* infection are unclear; however, experimental models have shown that *T. gondii* disrupts the structure and function of brain resident cells. This study investigated the effects of chronic *T. gondii* infection on proliferation and apoptosis of microglia, astrocytes, and neurons, and how these processes may contribute to shifts in cell population dynamics in the cortex, striatum, subventricular zone (SVZ), and hippocampus.

Female C57BL/6J mice were intraperitoneally infected with *T. gondii* (PRU strain, 5×10^4 tachyzoites) or PBS (control). Brains were collected at 1-, 3-, and 5-months post-infection (p.i., n=6 per group per time point). Brain sections (40 µm cryosections) were subjected to multiplex immunofluorescence staining using markers for parasite cysts (DBA Lectin), microglia (Iba1), astrocytes (GFAP), mature neurons (NeuN), newborn neurons (DCX), proliferation (Ki-67), and apoptosis (cleaved Caspase-3/7). Tissues were imaged using an Evident FV4000 confocal microscope, and acquired images were analysed with FIJI.

At one-month p.i., *T. gondii* induced a significant increase in microglial density within the cortex (unpaired t-test, p=0.0043); this was associated with an increase in proliferating cortical microglia (Ki67+ Iba1+). In contrast, no changes in astrocyte density or proliferation were observed in the cortex. In the striatum, microglial and astrocyte density did not differ significantly between infected and control groups. No differences were observed in the density of mature neurons or newborn neurons within the SVZ or hippocampus. Similarly, the number of proliferating newborn neurons did not differ between infected and control groups.

These preliminary findings suggest that during chronic *T. gondii* infection, microglia density and proliferation are altered in a region-specific manner, whereas changes in astrocytes and neurons were not observed.

Poster

Spatial mapping of microglia and astrocyte subtypes in experimental autoimmune encephalitis

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Multiple sclerosis (MS) is one of the most prominent and debilitating autoimmune diseases affecting the central nervous system (CNS). Despite the vastly increasing population and major uprising in incidences, there are no cures for this disease. Underpinning the pathogenesis of MS are immunological assaults on the protective myelin sheath surrounding nerve fibres. Microglia, the resident immune cells of the CNS, are thought to play a role in the immunopathogenesis of MS; however, microglia are a heterogeneous population, and it is not known which populations lead to the progression and potentiation of MS. Our study will investigate the spatial heterogeneity of microglia and astrocyte morphologically-defined subtypes in the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). This will enable us to determine the phenotype of specific microglia and astrocyte morphotypes and understand their spatial relationship with degenerating neurons, regions of demyelination, and T cell infiltration. This will be achieved through cryotomy sectioning of EAE mouse brains at 3 stages: pre-clinical, early disease, and peak disease. Brain sections will undergo immunostaining with antibodies to identify brain resident cell types (microglia, astrocytes, and neurons), myelin, and infiltrating encephalitogenic T cells. Tissues will be imaged using a confocal microscope, and FIJI/Image J, R, and QuPath will be used to analyse cell density, morphology, and spatial interactions. Statistical analysis will be performed using GraphPad Prism. Overall, this project aims to understand how heterogeneous microglial and astrocyte morphotypes are involved in disease progression.

Poster

Targeting IL-6 Trans-Signalling Reduces Inflammation in ALS Models

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by chronic inflammation in both the central nervous system (CNS) and peripheral tissues. Interleukin-6 (IL-6) has been implicated in ALS pathology; however, IL-6 exhibits both anti-inflammatory and pro-inflammatory functions. Notably, IL-6 trans-signalling possesses pro-inflammatory properties and is emerging as a key contributor to neuroinflammation during neurodegeneration. In this study, we aimed to characterize the expression of the IL-6 trans-signalling pathway in ALS mouse models and investigate its role in ALS protein aggregate-mediated inflammation in microglia and peripheral immune cells. Our results revealed that the protein expression level of a key IL-6 trans-signalling component, soluble IL-6 receptor (sIL-6R), was significantly increased in the spinal cord and tibialis anterior (TA) muscles of both SOD1G93A and rNLS8 TDP-43 transgenic mice. Additionally, using mouse primary microglia, human monocyte-derived microglia-like cells (MDMi), and blood peripheral immune cells, we demonstrated that recombinant TDP-43 protein elicits robust pro-inflammatory cytokine responses, including IL-6, TNF-α, IL-23, and MCP-1. These responses were attenuated when treated with a specific IL-6 trans-signalling inhibitor, sgp130Fc. Our findings suggest that the TDP-43-induced inflammatory response is, in part, IL-6 trans-signalling-dependent and highlight the role of IL-6 trans-signalling as a potential driver of chronic inflammation contributing to ALS pathology. These results support IL-6 trans-signalling as a promising therapeutic target for mitigating inflammation and slowing disease progression. Future research should explore the broader implications of modulating IL-6 trans-signalling in ALS.

Poster

Complement C3a receptor signalling constrains A1 astrocyte induction in motor neuron disease

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Motor neuron disease (MND) is a fatal condition characterised by the progressive and widespread degeneration of motor neurons. Dysregulation of the complement system and correlating glial activation is observed in MND. Recently, neurotoxic astrocytes that acquire a reactive 'A1' state have been identified as a key driver of MND pathophysiology. Previously, our laboratory has demonstrated that the receptor for the complement activation peptide C3a, C3aR is neuroprotective in the SOD1G93A MND mouse model, and is expressed by astrocytes. This project therefore aimed to characterise the potential role of the C3a-C3aR axis on neurotoxic astrocyte activation in MND. We utilised immunohistochemistry to confirm expression of C3aR on astrocytes in both WT and SOD1G93A mice in the early to mid-symptomatic stages of disease. To characterise the functional role of C3a in astrocyte activation, we treated primary and immortal mouse astrocytes with the immune stimulator lipopolysaccharide or TDP-43Q331K, along with recombinant mouse C3a. We employed qRT-PCR to measure A1 genetic markers, which revealed that C3a treatment constrained A1 expression in immune-primed astrocytes. Finally, we quantified A1 astrocyte expression in WT, C3aR-/-, SOD1G93A, and SOD1G93A x C3aR-/- mouse spinal cords. Genetic deletion of C3aR in SOD1G93A mice correlated with an acceleration of widespread A1 astrocyte induction, validating to an in-vivo role of C3aR in A1 induction. Together, our results show a protective role for the complement C3a-C3aR axis in MND, by diminishing A1 astrocyte induction

Poster

Targeting CXCR2 to Modulate Neutrophil-Mediated Inflammation in Motor Neuron Disease

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Background: Motor neuron disease (MND) is a progressive neurodegenerative disorder characterised by the loss of upper and lower motor neurons. Immune activation plays a critical role in MND pathogenesis, with increasing evidence implicating the involvement of neutrophils and their chemokine signalling pathways. CXCR2, a chemokine receptor involved in neutrophil recruitment, is associated with various inflammatory diseases, however, its role in MND remains unexplored. This study used SOD1G93A mouse model to investigate neutrophil involvement in MND and evaluate the therapeutic potential of CXCR2 antagonism.
Methods: Neutrophil number and activity were assessed in SOD1G93A and wild-type (WT) controls using automated haematology, immunohistochemistry, and ELISA. SOD1G93A mice were treated with a CXCR2 antagonist at the "pre-onset" stage. Neuroprotective effects were evaluated through assessments of motor performance, survival and neuropathology. LC-MS/MS analysis was used to determine drug distribution in peripheral and CNS tissues.
Results: SOD1G93A mice showed no significant changes in peripheral neutrophil counts during early disease stages. However, circulating neutrophils significantly increased at end-stage in SOD1G93A mice compared to WT controls. Immunohistochemistry showed increased neutrophil infiltration in SOD1G93A skeletal muscle, with elevated levels of neutrophil-derived degranulating enzymes as measured by ELISA. Despite these findings, CXCR2 antagonism did not improve motor performance, survival, or neuropathological outcomes compared to vehicle-treated mice. LC-MS/MS analysis revealed sustained plasma and muscle levels of the drug but lower CNS bioavailability.
Conclusions: Neutrophil activation increases with MND progression in SOD1G93A mice, suggesting a potential role in disease pathology. However, CXCR2 antagonism did not confer neuroprotective effects,

potentially due to limited CNS drug exposure or the involvement of alternative inflammatory mechanisms. Future studies should explore compounds with improved CNS bioavailability and further investigate other functional roles of neutrophils as therapeutic targets.