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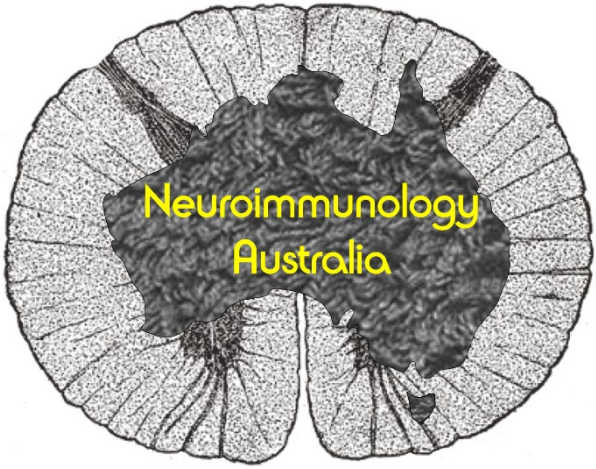

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POSTER abstracts

A role for perivascular astrocytes in the early trafficking of platelets across the blood brain barrier during neuroinflammation.

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Our laboratory has generated proof-of-concept that platelets are a substrate of neurodegeneration in multiple sclerosis (MS). Using the experimental autoimmune encephalomyelitis (EAE) model, we demonstrated early neuron-platelet associations and the efficacy of platelet-targeting in modifying disease course and in promoting remyelination and functional restoration. These observations imply the existence of mechanisms underlying the specific targeting of neurons by platelets and platelet trafficking across the blood brain barrier (BBB). This study aimed to identify signals driving platelet infiltration and the route of entry of platelets across the BBB. We used two EAE variants, namely the MOG_{35-55}-induced C57BL/6 and the $PLP_{178-191}$-induced SJL/J variants, which differ by the absence of requirement for pertussis toxin (PTx) in the SJL/J mice for disease induction. Controls were sham-injected mice. The focus was on the pre-symptomatic stage (4 to 9 days post-immunization [dpi]). Approaches included quantitative confocal microscopy with IMARIS analysis of astrocytes and correlative light and electron microscopy (CLEM) for platelet identification. We demonstrate early (4dpi) reactive platelet accumulation on endothelial cells and specific platelet-perivascular astrocyte end-feet association in both EAE variants and in grey and white matter. Platelets traverse rather than circumvent end-feet (9 dpi) and remain associated with astrocyte processes and cell bodies. These interactions were not observed with other glial cells, or in controls. We also demonstrate fibrinogen leakage into the parenchyma and neuronal expression of the fibrinogen receptor. We propose a mechanism whereby platelet targeting of neurons is triggered by BBB leakage and fibrinogen/fibrinogen receptor interaction. This drives platelet infiltration via a process in which astrocytes play a pivotal role. These data are relevant to the generation of platelet-targeting therapeutical strategies.

Pro-remyelinating properties of the anti-convulsant Valproic acid in the cuprizone MS model

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Multiple sclerosis (MS) is a prevalent immune-mediated disorder affecting the central nervous system, characterized by demyelination and neuronal damage. Current therapeutic strategies predominantly target the immune system, leaving a critical gap in therapies aimed at promoting myelin regeneration. Preliminary data and recent findings suggest a potential role of the non-neuronal gamma-aminobutyric acid (GABA) system in myelin repair, prompting investigation into drugs that modulate GABA availability. Our study explores the pro-remyelinating effects of Valproic acid (VPA), an FDA-approved anti-convulsant known to increase GABA bioavailability, in a murine cuprizone-induced model of MS. Using a reversible demyelination paradigm, 72 C57BL/6 mice were subjected to cuprizone diet for 4 weeks followed by a return to standard diet. Subsequently, mice were randomly assigned to receive daily intraperitoneal injections of either saline (control) or Valproic acid (200mg/kg) for 2 or 4 weeks. Clinical scores, locomotor skills (open field and Rotarod tests), and histological assessments were conducted to evaluate the effects of VPA on myelin regeneration.

VPA treatment accelerated clinical recovery, improved locomotor skills, and hastened myelin recovery. Gene expression studies revealed increased levels of myelin-associated genes, including myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), oligodendrocyte transcription factor 2 (OLIG2), and proteolipid protein 1 (PLP1), in VPA-treated mice compared to controls. Histological examinations using Luxol Fast Blue staining confirmed increased myelin recovery in drug-treated mice, while immunostaining for OLIG2 and PLP demonstrated enhanced oligodendrocyte activity. Additionally, VPA treatment was associated with reduced microglial polarization, as indicated by decreased immunoreactivity for the microglial marker IBA1.

This study elucidates the previously unrecognized pharmacological properties of VPA in myelin-producing cells, highlighting its potential as a repurposed therapy for MS. The findings underscore the importance of targeting non-neuronal GABAergic pathways in promoting myelin repair and highlight VPA as a promising candidate for further clinical investigations in MS treatment strategies.

Transcriptional regulation governing Th17 cells in experimental CNS autoimmunity

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Background: Th17 cells mediate pathology in mouse models of neuroinflammation and MS. Th17 cells are heterogeneous, consisting of non-pathogenic and pathogenic subsets with distinct roles in driving autoimmunity. Transcriptional mechanisms governing these distinct Th17 subsets are unclear, and may present targets for the treatment of MS.

Objective: We aimed to gain insight into transcriptional regulation governing pathogenic and non-pathogenic Th17 cell states in experimental autoimmune encephalomyelitis.

Methods: A novel strategy to specifically isolate pathogenic and non-pathogenic Th17 cells from EAE mice for RNA-seq was utilized. We generated a novel mouse line with a targeted knock out of a transcription factor in T cells, in order to assess the impact on the regulation of both pathogenic and non-pathogenic Th17 cells.

Results: Comparing pathogenic and non-pathogenic Th17 cell transcriptomes, we observed the gene encoding the transcription factor RUNX2 to be specifically increased in pathogenic Th17s. Flow cytometry analysis validated this at the protein level. Mice with a targeted T cell-specific deficiency of Runx2 displayed heightened severity of EAE, accompanied by an increased pathogenic Th17 cells infiltrating the CNS. Th17 priming in the spleen was also skewed towards pathogenic than non-pathogenic phenotypes in the absence of RUNX2.

Conclusion: Th17 cells express RUNX2 and this increases polarisation of these cells to a non-pathogenic phenotype in EAE. Future studies to understand the mechanisms that underpin this and to modulate this system for therapeutic benefit in neuroinflammation are required.

Impact of four COVID-19 vaccine boosters in people with multiple sclerosis: enhancing immune response and cross-variant protection

Rashmi Gamage, Avani Yeola, Samuel Houston, Vicki Maltby, Marzena Pedrini, Linh Le- Kavanagh, Vera Merheb, Kristy Nguyen, Fiona Xz Lee, Susan Walters, Marinda Taha, Annmaree O'connell, Vilija Jokubaitis, Angie Roldan, Mastura Monif, Helmut Butzkueven, Sandeep Sampangi, Alison Craig, Todd Hardy, Michael Barnett, Allan Kermode, Chris Dewyer, Tomas Kalincik, Simon Broadley, Stephen Reddel, Sudarshini Ramanathan, Jeannette Lechner-Scott, Anneke Van der Walt, Fabienne Brilot

Background: High-efficacy disease-modifying therapies (HE DMT) affect COVID-19 vaccine responses in people with multiple sclerosis (pwMS). Amid falling COVID-19 vaccine rates, we are studying if extra boosters, beyond the initial two doses, can enhance Ab levels and cross-reactivity against new variants.

Objective: Assess vaccine efficacy in generating spike Ab titers and cross-reactivity against Omicron variants.

Methods: Spike Ab titers were assessed in Spike Ab-seropositive pwMS (n=164) receiving HE DMT (Ocrelizumab, Natalizumab, Siponimod, Fingolimod, Alemtuzumab, Ofatumumab, n=122), platform DMT (P DMT, Dimethyl Fumarate, Teriflunomide, Glatiramer Acetate, Interferons-b, n=20), and cladribine (n=22) at 1 month post dose-2 and 1-6 month post dose-4. Booster effect on titers and cross-reactivity were determined on ancestral D614 and Omicron variants (BA.1, BA.2, XBB1.1, XBB1.5 and EG5).

Results: Additional vaccine doses (2 to 4) increased Spike Ab titers in Spike Ab-seropositive pwMS across vaccine types (BNT162b2 or ChAdOx1-S), DMT, and variants. Magnitude of this increase varied by treatment and variant. Smallest percentage rise from 2 to 4 doses occurred in the HE DMT group and against newer Omicron variants like XBB1s and EG5, while the largest increase was seen in older variants (D614 and the original Omicron BA.1). Highest titers were observed at 1 month post dose-4 in the P DMT group, with an overall 4.85 ± 2.9 SD-fold increase in titers against all variants compared to the HE DMT group (p=0.01). Older variants (D614, BA1, and BA2) displayed the highest increase compared to recent ones (XBB1s or EG5). Although DMT affects Spike antibody levels, the ability of antibodies to recognize multiple variants (cross-reactivity) stayed consistent (p=0.8) across groups, indicating uniform variant recognition after 4 doses.

Conclusion: Despite lower Spike Ab levels in pwMS on HE DMT after 4 vaccine doses, regular COVID-19 boosters benefit all pwMS, recognising emerging Omicron variants, ensuring protection, and reducing illness rates.

Exploring Brain Organoids as a Promising Model for Neuroinflammatory and Neurodegenerative Diseases

Romal Stewart

UQ Centre for Clinical Research, University of Queensland, Brisbane

Neurodegenerative and neuroinflammatory diseases present considerable global health challenges, contributing significantly to mortality rates worldwide. Despite their prevalence, many of these conditions lack effective treatment options, a consequence of our incomplete understanding of their pathophysiology. This knowledge gap arises, in part, from the inherent difficulties in accessing the human brain for comprehensive study. Traditional research models, such as in vitro cell monocultures and animal experimentation, often fall short in accurately reproducing human disease pathology and pharmacological responses.

Brain organoids offer a novel and promising approach to studying neuroinflammatory and neurodegenerative diseases. These three-dimensional neural cell cultures, derived from induced pluripotent stem cells, demonstrate the capacity to closely mimic the intricate developmental stages of the human brain. Dr. Stewart's presentation will delve into the substantial potential of brain organoids as a research tool, with particular emphasis on their ability to replicate the aberrations observed in Alzheimer’s disease.

Regulatory T cells are neuroprotective and facilitate DRG sensory neuron outgrowth

Jessica Hayes, Sandra Fok, Renee Whan, Gary Housley, Gila Moalem-Taylor

The University of New South Wales

Regulatory T cells (Tregs) are potent anti-inflammatory CD4+ T cells that are critically involved in maintaining immune homeostasis. Recent studies have demonstrated that enhancing Tregs in animal models of neuropathic pain attenuates pain behaviours, whereas depleting their population exacerbates painful behaviours, making Tregs a potential therapeutic target. Chemotherapy-induced peripheral neuropathy is a form of neuropathic pain caused by chemotherapeutics, such as paclitaxel (PTX). These drugs damage dorsal root ganglia (DRG) sensory neurons from their axon terminals to their somata, inducing neurotoxicity and neuropathic pain symptoms. Here, we investigated whether Tregs can interact with and protect DRG sensory neurons from the neurotoxic effects of PTX. Using 24-hour live cell imaging, we found that activated Tregs are attracted to DRG sensory neurons and facilitate neurite outgrowth, increasing their mean neurite length ($p=0.0417$), total neurite length ($p=0.0062$) and number of nodes ($p=0.0220$). Furthermore, Tregs protected the neurons from the inhibitory effects of PTX on neurite outgrowth ($p=0.0178$). These effects could be mediated by the milieu of factors Tregs produce, such as amphiregulin and IL-35. Amphiregulin is a growth factor that induces cell proliferation and differentiation and has been shown to facilitate neuronal outgrowth. We found that blocking the amphiregulin receptor with AG1478 inhibits DRG axonal outgrowth when co-cultured with Tregs ($p=0.0202$) but not when neurons were cultured on their own ($p=0.3992$). IL-35, an anti-inflammatory cytokine, has been shown to reduce pain behaviours in animal models of neuropathic pain when administered intrathecally. We found that 25ng/mL IL-35 had a mild protective effect on PTX-treated DRG sensory neurons ($p=0.0561$). Overall, we demonstrate for the first time that Tregs are attracted to DRG sensory neurons in vitro, facilitating their outgrowth and protecting them against the neurotoxic effects of PTX. These findings support a neuroprotective role of Tregs beyond their effects on the immune system.

More Efficient Complement Activation by Anti-Aquaporin-4 Compared With Anti-Myelin Oligodendrocyte Glycoprotein Antibodies

Magdalena Lerch[1], Kathrin Schanda[1], Eliott Lafon[1], Reinhard Würzner[1], Sara Mariotto[2], Harald Hegen[1], Kevin Rostasy[3], Thomas Berger[4], Doris Wilflingseder[2], Romana Höftberger[4], Markus Reindl[1]

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Background: Autoantibody mediated neurological autoimmune diseases comprise a broad spectrum that is rapidly evolving. Antibodies against aquaporin 4 (AQP4-IgG) are found in most patients with neuromyelitis spectrum disorder (NMOSD), whereas antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG) are linked to MOG-IgG associated disease (MOGAD). Both MOG-IgG and AQP4-IgG can induce complement-dependent cytotoxicity (CDC). CDC is a main pathological hallmark in NMOSD, whereas the role of complement activation in MOGAD is less clear. Therefore, we aimed to analyse CDC induced by AQP4-IgG and MOG-IgG in human patient serum samples.

Methods: A cell-based assay using different MOG isoforms or AQP4-M23 was utilised. Cells were incubated with human MOG-IgG or AQP4-IgG positive serum together with human complement and CDC was measured with a lactate-dehydrogenase assay, to quantify antibody mediated cell damage. Furthermore, the terminal complement complex (TCC) was quantified and analysed by flow cytometry and immunocytochemistry.

Results: AQP4-IgG positive serum samples stimulated higher CDC and TCC levels than MOG-IgG positive sera, but both showed a dependency on antibody titres. Moreover, AQP4-IgG induced higher TCC cell surface assembly than MOG-IgG. Immunocytochemistry revealed deposition of activated complement products on different MOG isoforms and AQP4, demonstrating complement activation on a cellular level.

Conclusion: Both MOG-IgG and AQP4-IgG induced CDC in a titre-dependent manner. However, AQP4-IgG showed markedly higher levels of CDC compared with MOG-IgG. This further highlights the role of complement in NMOSD, whereas its importance in MOGAD is less pronounced.

Multi-dimensional analysis of MOGAD patients uncovers a unique autoimmune landscape

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Introduction: Although immunosuppression is recommended in myelin oligodendrocyte glycoprotein antibody-associated disorder (MOGAD), optimal and duration of treatment at first presentation and over the course of the disease remain uncertain, and may be influenced by a deeper understanding of MOGAD aetiology

Objectives/Aims: To identify the autoimmune landscape and mechanisms responsible for MOGAD aetiology

Methods: Peripheral Blood Mononucleated Cells (PBMCs) of MOGAD patients at different disease stages and controls were analysed by HIVE CXL single cell RNA sequencing, 10X Genomics Chromium controller followed by Illumina HiSeq 3000/4000 sequencing, and 25-parameter flow cytometry

Results: In contrast to healthy controls, MOGAD patients displayed an additional B cell cluster that expressed CDC20 and SPC25 genes indicative of highly proliferative plasmablasts. Pathway analysis by REACTOME revealed that, in MOGAD patients, the adaptive immune system, signal transduction, neurotransmission, and vesicle-mediated transport were highly upregulated. However, although the comparison of the global distribution of peripheral immune cell populations did not show significant changes between acute and remission MOGAD stages, there was an abnormal B cell maturation relationship between naïve B cells and plasmablasts at onset compared to remission. Interestingly, clonotype assignment of B cells revealed that the remission paediatric MOGAD case had the lowest proportion of unique clonotypes (89.1%), but the greatest relative abundance of medium and large clonotypes

Conclusion: Our data suggest that the B cell lineage and its maturation underly MOGAD pathogenesis. MOGAD specific immune profile could facilitate efficient treatment decision making and patient management.

Understanding pathogenic Th17 cell recruitment to the inflamed nervous system

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Pathogenic Th17 (pTh17) cells have been shown to promote autoimmunity via the expression of inflammatory cytokines, particularly IL-17A and GM-CSF. Due to their prominent role in driving inflammation, pTh17 cells are an attractive potential target for treatment of autoimmune diseases such as multiple sclerosis (MS), an inflammatory demyelinating autoimmune disease of the central nervous system (CNS) which we model in mice utilising experimental autoimmune encephalomyelitis (EAE). Therapeutics that can specifically block recruitment of pTh17 cells to the inflamed CNS may be more effective than current disease-modifying therapies used in MS. However, the molecular mechanisms used by pTh17 cells to migrate into the inflamed CNS during EAE and MS are only partially known. Eighteen distinct chemokine receptors differentially support leukocyte migration in vivo and, to date, none of these in isolation has been shown to be essential to pTh17 cell recruitment to the inflamed CNS. In addition, there are many other receptors and ligands known to contribute to leukocyte migration.

We propose that pTh17 cells utilise combinations of migratory receptors to migrate to and infiltrate the CNS to drive disease in EAE and that the pathogenic subsets of these cells may also be identified by unique combinations of these molecules.

Thus, we have used single cell RNA sequencing to study all migratory molecules expressed by CNS-infiltrating CD4+ T cells in EAE to better understand pTh17 cell migration. We are interrogating high-dimensional migratory molecule signatures and cell trajectories of pTh17 cells that have invaded the CNS to identify combinations of migratory receptors that may represent novel therapeutic targets to treat MS.

APOE genotype is associated with a brain and peripheral immune signature independent of cognitive status and Alzheimer's disease pathology

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A mutation in the apolipoprotein E gene called ε4 (APOE4) is the biggest genetic risk factor for late onset Alzheimer's disease (AD). Proteome-wide changes independent of brain pathology and cognitive status remain unknown. Here, we investigated APOE4-associated proteome changes in people with AD, mild cognitive impairment (MCI), and no impairment. Patient clinical, APOE genotype, and cerebrospinal fluid (CSF) proteome data for 735 participants was sourced from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Supervised machine learning was used for proteome profiling to identify protein signatures and enrichment analyses were performed using NetworkAnalyst 3.0 and data from the Human Protein Atlas.

We found an APOE4-specific proteome signature across APOE4 carriers independently of cognitive status and AD pathology burden in the CSF, including total tau, phospho-tau, and amyloid-β 42. Importantly, this signature was associated with an increased risk of progression to clinical cognitive impairment over time. Proteins within the signature were enriched in brain regions including the caudate and cortex as well as brain cells including endothelial cells, oligodendrocytes, and astrocytes. Enriched peripheral immune cells included T cells, macrophages, and B cells. The APOE4-specific CSF proteome signature was independent of cognitive status and AD pathology burden and increased risk of progression to cognitive impairment over time. It was associated with a clear immune and inflammatory phenotype across the brain and periphery.

Our findings suggest that the APOE4 proteome signature is independent of AD-specific brain pathology and likely underlies APOE4 carriers' vulnerability to cognitive decline and AD as they age.

Interleukin-6 and interferon-alpha differentially regulate microglia function

Rovin Verdillo

The University of Sydney

Interleukin-6 (IL-6) and interferon-alpha (IFN-a) are two key cytokines that elicit distinct immune responses in the central nervous system (CNS). While IL-6 is produced in a wide variety of infections and mediates the innate immune response, IFN-a is produced primarily in viral infections and facilitates the antiviral response. Acute production of these cytokines is sufficient to resolve disease. However, prolonged or chronic production contributes to diverse neuropathologies. Recently, our (Hofer) lab has identified distinct phenotypes of microglia in transgenic mice with brain-targeted chronic production of IL-6 or IFN-a. While transcriptomics are considered the 'gold-standard' to delineate cellular and molecular mechanisms in disease, functional validation, although more challenging and thus often neglected, is necessary to bridge the gap between gene expression and biological outcome.

Here, I established an in vitro model that simulates chronic IL-6 and IFN-a exposure and conducted in vitro functional assays to measure migration and phagocytosis, two key microglia functions, on cytokine-treated primary murine microglia. Our chronic cytokine model verified the specificity of IL-6 and IFN-a signalling. Further, IL-6 treatment stimulated cell proliferation, migration and phagocytosis, whereas IFN-a treatment induced cytotoxicity and inhibited migration and phagocytosis. These findings demonstrate that IL-6 and IFN-a differentially regulate microglia function. Our results also validate our recent phenotypic data from in vivo microglia. Together, the findings suggest that during neuroinflammation, where IL-6, IFN-a, and many other cytokines are simultaneously present, microglia function is likely subject to the predominant cytokine present. This opens new potential avenues into identifying pathogenic mechanisms and developing treatment that is aimed at modulating these pathways.

Comparative effectiveness of autologous haematopoietic stem cell transplantation and immune-reconstitution therapies in relapsing-remitting multiple sclerosis (MS)

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Chemotherapy with autologous hematopoietic stem cell transplantation (AHSCT) shows several similarities with immune-reconstitution therapies in relapsing-remitting MS. These therapies have not previously been compared head-to-head. We have carried out pairwise comparisons of the effectiveness of AHSCT with cladribine and alemtuzumab.

Patients with relapsing-remitting MS from 7 AHSCT MS centres were combined with patients from MSBase. Included patients were treated with AHSCT, cladribine or alemtuzumab and had sufficient information recorded before and after the start of the treatments (baseline). Groups were matched on a propensity score derived from sex, age, disability score (EDSS), number of relapses 12 and 24 months before baseline, time from MS onset, the most effective prior therapy and geographic region. The groups were compared on annualised relapse rates (ARR), cumulative hazards of relapses and 6-month confirmed EDSS worsening and improvement.

The matched patients (134:562 AHSCT:cladribine, 143:283 AHSCT:alemtuzumab) had high mean disease activity (>0.8 relapses in the prior 2 years), mean EDSS 3-4, and were followed-up for a mean of 1.9-4.5 years. Compared to cladribine, AHSCT was associated with a lower risk of relapses (mean ARR±SD 0.05±0.28 vs. 0.16±0.39, respectively; hazard ratio 0.24, 95%CI 0.15-0.41), similar risk of EDSS worsening (hazard ratio 0.70, 95%CI 0.34-1.43) and higher probability of EDSS improvement (hazard ratio 2.19, 95%CI 1.31-3.66). Compared to alemtuzumab, AHSCT was associated with a lower risk of relapses (mean ARR±SD 0.04±0.23 vs.

0.09±0.21, respectively; hazard ratio 0.52, 95%CI 0.29-0.93), similar risk of EDSS worsening (hazard ratio 0.95, 95%CI 0.53-1.72) and higher probability of EDSS improvement (hazard ratio 2.03, 95%CI 1.23-3.34). 34% of patients treated with AHSCT experienced delayed complications, mainly infections. No treatment-associated deaths were reported.
Among patients with active relapsing-remitting MS and moderate disability, AHSCT is superior to cladribine and alemtuzumab in suppressing relapses and enabling recovery of neurological function.

Targeting centrally produced interferon-alpha to resolve brain disease

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Interferon-alpha is a cytokine essential for host antiviral responses. Elevated interferon-alpha does negatively impact the brain and can result in sickness behaviour and cognitive impairment, such as from viral infections, but also angiopathy and neuropathology, such as in Aicardi-Goutières syndrome (AGS). AGS is a genetic disease resulting in increased interferon-alpha levels in the blood and cerebral spinal fluid. Consequently, some AGS patients show similar symptoms with those with systemic lupus erythematosus (SLE), an autoimmune disease with increased blood interferon-alpha. Additionally, some AGS patients also develop brain disease encompassing cognitive and motor dysfunction. We hypothesised that the centrally produced interferon-alpha in AGS causes brain disease.

Using single molecular ELISA, we first confirmed significantly elevated concentrations of interferon-alpha in the cerebral spinal fluid, but not blood, of AGS patients but not SLE patients. By pairing blood interferon-alpha concentrations with MRI for angiopathy, we identified no correlation between the two factors. We used a mouse for AGS and orally treated the mice with baricitinib, an inhibitor of interferon signalling. Although interferon signalling was downregulated in the periphery, this did not occur in the brain, nor did it significantly improve survival. Next, intracerebroventricular injections of antisense oligonucleotides against the interferon receptor was given to the mice. Treated mice showed downregulation of interferon signalling in the brain along with improvements in features of neuropathology.

These results demonstrate centrally produced interferon-alpha mediates brain disease and targeting it is a therapeutic option for AGS. Moreover, these results implicate a damaging role of elevated interferon-alpha identified in the brains of aged individuals to various neurodegenerative diseases.

The clinical utility of metabolomics in paediatric neurological disorders: from bench to bedside

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Inflammation of the brain is increasingly recognised as important in encephalitis, but also neurodevelopmental, neuropsychiatric and neurodegenerative processes. The high mortality and morbidity rates of acute neuroinflammatory diseases has directed significant interest in the investigation of biomarkers to define neuroinflammation and explore mechanisms involved in the regulation of immune responses.

An untargeted cerebrospinal fluid (CSF) metabolomics study investigated a cohort of fourteen patients with acute encephalitis and age-matched non-inflammatory neurological disease controls (n=14). CSF metabolites were analysed using liquid chromatography coupled to high resolution mass spectrometry followed by subsequent multivariate and univariate statistical methods. Neopterin, tryptophan-kynurenine and nitric oxide pathways contributed to the discrimination between acute encephalitis and controls. An increase in CSF kynurenine/tryptophan ratio ($p<0.001$), asymmetric dimethylarginine/arginine ratio ($p<0.001$), and neopterin ($p<0.001$) strongly predicted neuroinflammation.

The alterations in metabolite profiles of human diseases often occur well before the signs of clinical symptoms. The preliminary discovery-based untargeted approach identified a useful panel for neuroinflammation. To translate the research data, we developed and clinically validated a targeted LC-MS/MS method for the quantification of CSF metabolites from the pterin, tryptophan-kynurenine and nitric oxide pathways. This method was applied to a cohort of 375 paediatric patients; primary inflammatory disorder group (n=90), and epilepsy group (n=114), were compared with three control groups including neurogenetic (n=76), neurodevelopmental disorders, psychiatric and functional neurological disorders (n=63), and headaches (n=32).

The study demonstrated decreased CSF kynurenic acid and kynurenic acid/kynurenine ratio in epileptic spasms, which may also represent a biomarker for steroid responsiveness. Moreover, we show that CSF neopterin, kynurenine, quinolinic acid and kynurenine/tryptophan ratio are

useful diagnostic and monitoring biomarkers of neuroinflammation. Early detection of inflammation is crucial to ensure efficient treatments and improved clinical outcomes. We present an inflammation panel integrated into healthcare showcasing direct translation into clinical practice.

Neuroinflammation in fetal growth restricted newborns

Kirat K. Chand

University of Queensland Centre for Clinical Research, Royal Brisbane & Women's Hospital, Brisbane.

Fetal growth restriction (FGR) is commonly caused by placental insufficiency, resulting in abnormal brain development. FGR is associated with poor neurodevelopmental outcomes such as learning and behavioural issues, and in severe cases cerebral palsy. Cellular development in the FGR brain during the perinatal period is not well characterised. In addition, no treatment exists to protect the FGR newborn brain. Using our pre-clinical pig model of FGR, we have characterised key cellular markers of neuronal and white matter structures, as well as broadly examining glial populations. We have shown early and persistent neuroinflammation is associated with brain injury including altered neuronal integrity, hypomyelination, glial activation and reduced neurovascular integrity in FGR newborns. Through characterising pathology and examining contributing pathways, we can now investigate potential therapeutic targets to improve brain outcomes in this vulnerable population.

Targeting STING, TOLLIP and the unfolded protein response to limit secondary injury post-TBI.

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Traumatic brain injury (TBI) is a major cause of death and disability worldwide with limited pharmacological interventions available to slow the inflammation and neurodegeneration that ensues post-injury. Our lab has demonstrated the cGAS-STING pathway as a key driver of neuroinflammation-mediated neurodegeneration in TBI and other neuropathologies. Known to be a regulator of type-I interferon production, there is increasing evidence for additional roles for STING in mediating cell death and ER-stress. Toll interacting protein (TOLLIP), is an endogenous negative regulator of TLR signaling with roles in misfolded protein trafficking. Recently, TOLLIP has been identified to regulate STING activity, by stabilising STING at its resting state on the ER.

This study aims to evaluate the role of TOLLIP in regulating STING and ER-stress activity in the CNS post-TBI. 10–12-week-old male C57Bl/6 mice were exposed to brain injury using the controlled-cortical impact model (CCI). 30-minutes post-injury, mice were intravenously administered a single 750nmol dose of the STING inhibitor, C-176 or saline (vehicle). Analysis was conducted 2 and 24-hours post-TBI (n=7-9).

Western blot analysis revealed a significant reduction in the expression of TOLLIP and total STING in the cortex 24h-post TBI and not 2h-post TBI in both C-176 (0.65±0.08) and vehicle-treated mice (0.65±0.10) when compared to sham (1.55±0.15). C-176-treated mice alone exhibited significantly increased phosphorylation of the unfolded protein response (UPR) regulator IRE1-α (6.14±1.24) compared to both sham (1.00±0.12) and vehicle-treated TBI mice (1.37±0.28). Striatal mRNA expression levels of its downstream mediator Xbp1 were also elevated at 24h-post TBI when compared to sham mice (C-176=1.24±0.11; sham=1.03±0.08).

Together these findings suggest TOLLIP and its stabilising activity on resting-state STING is lost-post TBI with the pharmacological inhibition of STING increasing activation of the IRE1-XBP1 branch of the unfolded protein response. This study provides novel mechanistic insight into the regulation and activity of STING post-TBI in mice.

High fat diet consumption and social instability stress impair stress adaptation and maternal care in C57Bl/6 mice

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Persistent stress and inflammatory environmental exposures during pregnancy can have long-term implications for shaping the future health of offspring. Animal models have improved our understanding of the role of stress and inflammation on maternal health and prenatal development. However, previous models have not studied the effects of multiple sources of environmental stress during pregnancy.

Here, we developed a novel model of maternal stress which include chronic high-fat diet (HF+) consumption and 6-weeks of social stress instability stress (SIS+) exposure. The SIS paradigm presents as a chronic and unpredictable form of social stress in rodents within group-housed environments. Briefly, we observed HF+/SIS+ females demonstrated weight-gain and glucose intolerance following 8-weeks of diet administration despite SIS exposure. They exhibited elevated plasma adrenocorticotropic hormone and corticosterone levels following the end of SIS exposure while control groups demonstrated significant reductions in both hormones following SIS. Anxiety-like behaviours remained unchanged prior to inducing pregnancy. However, nest building testing revealed a trend of poorer nest quality compared to HF- groups. Lastly, these HF+/SIS+ mice demonstrated significant postpartum maternal neglect towards offspring.

Thus, HF diet and SIS exposure place a significant burden on the maternal stress response system, resulting in a maladaptive response to stress and diminished parental investment and negative postpartum behaviours towards offspring.

Complement C5aR1 drives NLRP3 inflammasome mediated neuropathology in Parkinson's disease preclinical models

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Parkinson's disease (PD) is a rapidly growing neurological disorder marked by dopaminergic neuron loss in the substantia nigra and α-synuclein aggregates. Microglia-driven immune-mediated neuroinflammation is a known PD contributor. We previously showed that inhibiting the microglial NLRP3 inflammasome prevents α-synuclein pathology in mice. However, the role of the innate complement system remains unexplored.

Analyzing public transcriptomic and proteomic data, we found widespread complement upregulation in PD patient brains at dopaminergic neuron loss sites and in peripheral blood. Fibrillar α-synuclein activates complement in human plasma, with proteomic studies highlighting C5a as a key upregulated factor in PD patient serum, along with increased C5aR1 expression in human and mouse microglia.

In three PD models, complement and microglial C5aR1 were upregulated following dopaminergic degeneration in the nigrostriatal pathway. Genetic deletion of key complement effectors (C3, C5, and MAC) in a neurotoxin-based PD model revealed a critical role for C5aR1 in neurodegeneration. To test therapeutic potential, we examined the C5aR1 inhibitor PMX205 in reducing neuropathology and motor dysfunction in the 6-OHDA model. Using mass spectrometry imaging (MALDI-MSI), we correlated behavior, microglial activation, and dopamine distribution, showing neuroprotection in 6-OHDA-injected human C5aR1 knock-in mice and wild-type mice orally dosed with PMX205.

Testing PMX205 in a 12-month α-synuclein fibril PD model, and using F18-DPA-714 PET/CT imaging to visualize microglial activation, we found that both prophylactic (0-12 months) and therapeutic (4-12 months) oral PMX205 administration improved motor deficits, dopaminergic neurodegeneration, and F18-DPA-714 signals.

Mechanistically, microglial NLRP3 inflammasome activation was impaired in the absence or inhibition of C5aR1 signaling. Complement activation and persistent C5a generation by fibrillar α-synuclein in the PD brain contribute to microglial NLRP3 inflammasome activation, exacerbating pathology. Targeting C5aR1 with inhibitors could reduce microglial-mediated neuroinflammation and slow PD progression.

ECR: Early career researcher or end of career in research?

Aakanksha Dixit

Doctoral studies represent a formidable challenge, and one might assume that after investing significant effort, the career path would become relatively straightforward. Each year, around 10,000 individuals embark on their PhD journeys, yet only about 25% of them ultimately continue within academia. Without proactive measures, this percentage is likely to dwindle further. Are we equipping our Higher Degree by Research (HDR) students with the essential skill sets required for gainful employment? Are we ensuring their success in a research-oriented career, should they choose that path? What if the current state of affairs in the research field discourages potential entrants?

These queries prompted us to organize a SWOT workshop for our final-year HDR students and early career researchers at the University of Queensland Centre for Clinical Research. The SWOT parameters were both individual and institute wide and what emerged from this exercise is a gradual depletion of our research community. While there were positive aspects relating to the institution and its collaborations with similar entities, the threats were primarily associated with the prevailing research landscape, marked by inadequate funding and government support. This prompts the question: Do we merely acknowledge the existing grievances, emphasizing their longstanding nature, or do we take steps to effect change within the research sphere, making it more inviting?

To understand this on a larger scale I would like to repeat the analysis with the audience present at the NIA Workshop 2024.

Poster Abstracts

Poster

Investigating autoimmunity in Multiple Sclerosis and Fibromyalgia pathologies

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Background: Multiple Sclerosis (MS) is a common non-traumatic disabling disease affecting young adults and characterised by chronic inflammation and demyelination. While MS is commonly classified as an autoimmune disease, the precise mechanisms responsible for the immune system's recognition of self-proteins as antigenic targets remain elusive. Fibromyalgia is the second most common "rheumatic" disorder associated with a chronic widespread pain syndrome. Both MS and fibromyalgia are chronic diseases with fluctuation over time, suggesting they could share similarities.

Objective: We aim to analyse the immunological characteristics to identify the spectrum of autoantigens that potentially contribute to the onset and progression of the diseases. Therefore, we could gain a better understanding of the disease's aetiology and a more precise diagnosis during its early stages.

Methods: We used mass spectrometry-based proteomics to compare the proteome signature of plasma from individuals with MS, fibromyalgia, and control, and identified significant changes in protein expression (confidence score $\geq$50, max fold $\geq$1.2, P value $\leq$0.05) functional pathways (Metascape, Panther, KEGG, STRING). In parallel, we assessed the antigen auto-recognition with IgG derived from each individual with dot blot assays and SDS-PAGE separations. In-gel digestion has been performed on the antigens pulled down with IgG.

Results: Across MS and Fibromyalgia samples, plasma-based proteomic analysis specifically identified a host of immune-related proteins and provided insight into immunological characteristics in both diseases. The co-immunoprecipitation of IgG and bound antigens showed proteins expressed selectively in some patients. Lastly, the dot blot assays revealed variable cross-reactivity across the samples.

Conclusion: MS and fibromyalgia exhibit various post-pathogenic changes commonly observed in multiple brain disorders. However, in-depth studies examining the immune cross-reactivity involving host and foreign antigens are needed to distinguish MS- and fibromyalgia-specific modifications and unravel crucial insights into disease pathogenesis.

Poster

Activation of microglial IL6 trans-signalling attenuates acute neuronal deficits following stroke

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Stroke is a leading cause of death worldwide, with survivors facing increased risk of disabilities, such as motor function impairment. In murine stroke models, IL6 levels rise in brain parenchyma during acute disease stages. The inhibition of this endogenous IL6 exacerbates post-stroke lesion size, while exogenous IL6 administration diminishes infarct volume and neuronal death. Although IL6 signalling evidently confers neuroprotective benefits post-stroke, the specific cell types activated by IL6 and responsible for inducing the neuroprotective environment remain elusive. Recent research suggests a correlation between IL6 trans-signalling activation in microglia and neuroprotection in a traumatic brain injury model. Therefore, we aim to study if stimulation of the IL6 trans-signalling pathway in microglia can replicate beneficial effects within the context of stroke. For this, we used the CX3CR1CreERT2xLgp130fl/+ mouse strain, which allows inducible microglia-specific IL6 trans-signalling activation through expression of Lgp130. Mice were subjected to a middle cerebral artery occlusion (MCAo) for 60min – a transient model of stroke. After 24-hours of onset, motoric deficits were assessed. Motor coordination was evaluated by the number of foot-faults in tapered beam test, while exploratory and anxiety-like behaviors were addressed by open-field test. Moreover, MRI scans were performed 4-days post-stroke to determine histological damage: lesion size and fractional anisotropy index in white matter tracts to monitor axon integrity. We observed a significant improvement in fine motor coordination (decreased number of foot-faults) in microglia Lgp130 mice. Also, the willingness to explore and anxiety levels in microglia Lgp130 mice reached similar values to sham group. Moreover, MRI scans showed significant reduction of infarct volume in microglia Lgp130 group (stroke:52.2mm3, stroke+ microgliaLgp130:15.4mm3, p-value=0.02), and a significant increase in axon integrity in hippocampal fimbria, corpus callosum, and anterior commissure tracts. Overall, our data suggests that IL6 trans-signalling activation in microglia can improve functional and structural outcomes during acute stages post-stroke.

Poster

Optimizing intravenous immunoglobulin treatment in inflammatory neuropathy

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Intravenous immunoglobulin (IVIg) is the cornerstone of treatment for the Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP). The former is an acute monophasic disease whilst the latter is more insidious in onset and often leads to a protracted disease course. Both result in damage to the peripheral nerves and nerve roots, causing paresis and sensory deficits that can result in severe disability or sometimes death. Numerous working mechanisms for the proven therapeutic effect of IVIg in these two immune-mediated neuropathies have been postulated. The common denominator among these is that high-dose IVIg treatment is required for an anti-inflammatory effect. The induction dose for both diseases is 2 g/kg over 5 days, with subsequent maintenance dosing for CIDP often set at 0.4 g/kg every 3-4 weeks. Yet, empiric guidelines or readily-available biomarkers to achieve and monitor these doses are currently lacking. Required dose regimens are highly variable, which leaves patients at risk for undertreatment as well as overtreatment with this expensive and scarce drug. For GBS the standard induction course seems insufficient for about 20-25% of the patients with poor outcome, despite timely treatment. We aimed to establish an understanding of the pharmacokinetics (PK) of IVIg and to find biomarkers that could influence or predict the PK of IVIg in patients with GBS or CIDP. We undertook a series of studies to get an understanding of the presumed important factors in immunoglobulin G (IgG) metabolism assessing: IgG glycosylation, genetic variation in relevant receptors, other proteins of potential influence and disease/demographic features. Data of 369 patients was analysed by means of non-linear mixed-effects modelling. This culminated in the first population-pharmacokinetic model of IVIg treatment in GBS, providing us with a new tool to predict the PK and thereby the pharmacodynamics of IVIg to start personalising treatment.

Poster

Impact of a high fibre diet on immune cells and the Multiple Sclerosis animal model

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Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system with an increasing global incidence rate. Previous studies demonstrated the linkage between inadequate dietary fibre intake and dysregulation of the immune response. It is believed that dietary fibre interact with the host microbiome and foster the production of short-chain fatty acids (SCFAs) through the fermentation process, which in turn recruits regulatory T-cells (Tregs) and mediates anti-inflammatory effects. However, the roles of fibre intake in MS remains unclear.

Female C57Bl/6 mice were randomly fed a control (AIN93G pallet), HF (20% cellulose and 20% guar gum) or zero-fibre (ZF, fibre-free AIN93G pallet) content diet with ad-libitum food access for 4 weeks. Next, EAE was induced by immunization with a standard protocols using the myelin-derived peptide MOG. Immune profiles were analysed by flow cytometry at 4-weeks after starting the diet (before immunization) and during EAE (40-days post-EAE-induction). Mice were monitored daily to assess clinical progression.

4-weeks of HF feeding led to a significant body weight difference compared to ZF cohorts due to energy density difference. Immuneprofiling of blood cells and spleen after 4 weeks on the diet revealed that percentages of pro-inflammatory Ly6Chi monocytes were increased significantly in the HF compared to the ZF group. On the contrary, there was a reduction in the frequency of Tregs in the HF group compared to ZF. Metabolomics data from plasma samples revealed a distinct pattern of metabolite profiles after HF feeding, especially tryptophan-derived metabolites. Following EAE induction, the HF groups showed a delayed onset of clinical EAE, but an overall higher disease severity compared to the ZF group. Immuneprofiling showed a reduction of pro-inflammatory monocytes in the periphery along with higher microglia activation in the CNS. Further analyses will be required to examine the mechanisms underlying these observations.

Poster

Exploring Soluble TREM2 Biology and the Role of Extracellular Vesicles in the Pathophysiology of Multiple Sclerosis

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Background: TREM2 (triggering receptor expressed on myeloid cells) is a critical signalling mediator for CNS repair and immune modulation. CI L Piccio has shown that TREM2 secreted (sTREM2) from myeloid cells is elevated in the cerebrospinal fluid (CSF) of individuals with Multiple Sclerosis (pwMS), making it a promising MS biomarker. However, the context and nature of sTREM2 are not fully understood. Extracellular Vesicles (EVs) derived from myeloid cells including microglia are potentially relevant to MS pathogenesis as it may contain immune modulators that could be contributors to neuroinflammation, neuroprotection and/or be relevant to the homeostasis of myelin. Studying the association of sTREM2 and EVs that take part in cell-to-cell signalling can provide novel insight into the biological mechanism of MS progression.

Objectives: To investigate the presence of TREM2/sTREM2 and its signalling proteins in EVs from cultured human macrophages. To study sTREM2/TREM2 regulation after macrophage activation with inflammatory stimuli such as LPS & POLY: IC which mimics pathological inflammation. Methods: EVs released from cultured macrophages of healthy controls were ultracentrifuged at 18,000 & 100,000 g-force to isolate large and small EVs respectively. ELISA and LC-TMS were performed on the samples to detect sTREM2 and its association with EVs. Isolation of EVs was confirmed by NTA, TEM and mass spec identification.

Results: ELISA analysis showed the level of sTREM2 in the macrophage culture media was reduced after ultracentrifugation, suggesting some sTREM2 is associated with EVs. LC-TMS showed TREM2/sTREM2 protein is associated with both large and small EVs. Finally, after stimulating the macrophage with either LPS or POLY: IC in vitro (1ug/ml), sTREM2 levels in the culture media were depleted.

Conclusions: This study is the first to show evidence that TREM2/sTREM2 is associated with EVs, enhancing our understanding of this protein's function in relation to its potential as a biomarker.

Poster

Evaluation of the ability of anti-inflammatories to restore K+ homeostasis during neurodegeneration

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Neuroinflammation and glial cell dysfunction are associated with neurodegenerative diseases and are central to disease pathology. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease associated with a specific loss of motor neurons (MNs) leading to motor dysfunctions. The SOD1G93A mouse model of ALS is accompanied by increased levels of neuroinflammation, reactive astrocytes, and impaired ability of astrocytes to maintain K+ homeostasis specifically in the primary motor cortex. This study aims to evaluate the therapeutic effect of phytosomal curcumin, a cytokine suppressive anti-inflammatory drug, in ameliorating ALS disease progression and symptoms in SOD1G93A mice. Using various behavioural tests, electrophysiological recordings, imaging, and molecular biology techniques we assessed motor functions, dysregulation of K+ ion homeostasis, and neuroinflammation levels. Our preliminary results indicate significant motor deficits in SOD1G93A mice that were fed with a normal diet; however, a curcumin-enriched diet led to a slight (yet insignificant) increase in the latency to fall and a decrease in the time to traverse the beam in SOD1G93A mice, suggesting that curcumin had a limited effect on alleviating motor deficits during ALS progression.

Poster

The clinical relevance of MOG antibody testing in cerebrospinal fluid

Molly Reynolds, Irene Tan, Stephen Reddel FRACP PhD, Benjamin Trewin FRACP, Magdalena Lerch, Snehal Shah, Nigel Wolfe FRACP, Katherine Buzzard FRACP PhD, Jeannette Lechner-Scott FRACP PhD, Marzena Fabis-Pedrini, Anthony Fok, Nevin John, Chris Kneebone, Con Yiannikas FRACP MD, David Brown FRACP PhD, Allan Kermode FRACP PhD, Russell C Dale MRCP PhD, Fabienne Brilot PhD, Sudarshini Ramanathan FRACP PhD on behalf of the Australasian MOGAD Study Group

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Background: Myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) defines a distinct demyelinating disorder. The clinical relevance of MOG-immunoglobulin G (MOG-IgG) in cerebrospinal fluid (CSF) without corresponding seropositivity (CSF-restricted MOG-IgG) remains unclear.

Methods: The national diagnostic and clinical database for the Australasian MOGAD Study Group was reviewed from 2018 to 2024. Patients were diagnosed as MOG-IgG positive in serum or CSF using a flow cytometry live cell-based assay.

Results: Over six years of testing in a national referral centre, 1127 patients had paired CSF and serum samples tested. 59/111 (53.2%) of CSF samples in seropositive patients demonstrated concurrent CSF positivity for MOG-IgG, while 52/111 (46.8%) seropositive patients were antibody-negative in paired CSF. 1009/1016 (99.3%) seronegative patients were negative in paired CSF samples, and 7/1016 (0.7%) of seronegative cases had a CSF-restricted MOG-IgG profile. Of these seven patients, four had an alternate diagnosis confirmed, including three with multiple sclerosis and one with central nervous system vasculitis. The remaining 3/7 patients presented with longitudinally extensive transverse myelitis, classified as a high-risk phenotype for MOGAD, although one was diagnosed with a likely parainfectious myelitis and another as transverse myelitis associated with Sjogren's syndrome.

Conclusion: CSF-restricted MOG-IgG profile is rare, over half of these patients have an alternate non-MOGAD diagnosis confirmed. We strongly recommend the use of serum as the biospecimen of preference in testing for MOG IgG. We additionally urge caution in the interpretation of CSF-restricted MOG-IgG in unselected patients who do not have clinical, radiological or ancillary investigations consistent with MOGAD.

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Poster

IL-6 trans-signalling rescues dopaminergic neurons following traumatic brain injury

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Traumatic brain injury (TBI) can increase an individual's likelihood for developing Parkinson's disease by up to 50%, representing a significant non-genetic risk factor. Previous studies have reported that TBI causes loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), a structure affected during Parkinson's disease pathogenesis. Here, we used a unilateral controlled cortical injury model of TBI in mice and delivered injury to the motor cortex, a brain region often affected by TBI. We found that TBI resulted in significant loss of dopaminergic neurons in the substantia nigra, a site very distal to the initial injury location. Next, we investigated whether our recently discovered neuroprotective phenotype of repopulating microglia, induced by their replenishment shortly after injury (Willis et al., 2020), could mitigate dopaminergic neuron loss after TBI. Indeed, we found that repopulating microglia reduced the loss of dopaminergic neurons after TBI. Further, we found that such benefits were dependent upon microglial derived IL-6 signalling. Last, stimulation of IL-6 signalling using the designer cytokine Hyper IL-6 shortly after TBI was able recapitulate these benefits to improve dopaminergic neuronal survival, without the need to turnover microglia. Taken together, these results demonstrate the acute vulnerability of dopaminergic neurons in the SNpc, a site distant from the initial impact, and the protective effects of microglial IL-6 trans signalling thereon.

Poster

Understanding the glial mediated toxicity in Alzheimer's disease

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Alzheimer's disease is a multifactorial, irreversible neurological disorder, showing a complex pathology, such as amyloid-beta plaques (Aβ), hyperphosphorylated tau, and loss of cholinergic neurons and synapses of the basal forebrain resulting in cognitive impairment and memory loss. T-cell independent chronic neuroinflammation, described by increased glial reactivity, is an early pathological process evident in many neurodegenerative diseases, including AD. In order to slow down the progression of age-related neurodegenerative diseases, we believe that investigating the interactions between different contributing factors, such as neuroinflammation, basal forebrain cholinergic cell loss and tau hyperphosphorylation, in combination with age, could lead to new targets and disease-modifying interventions.

For behavioural test and histological analysis, we used mice aged 6 months (young) and 12-months (adult) wild type control, GFAP-IL6 mice that are overexpressing IL-6 under the GFAP promoter, Tau58/2 mice that are translating human 0N4R tau isoform with the P301S mutation in the brain, and GFAP-IL6XTau58/2 (on the same genetic background C57BL/6) mice that are crossed for both inflammation and Tau protien hyperphosphorylation.

Our findings demonstrated a potential direct effect of chronic glial reactivity on glial and neuronal cell numbers, altered cellular morphology, and declining cellular function resulting in both cognitive and motor function decline with ageing. The adult GFAP-IL6 and GFAP-IL6XTau58/2 mice showed an increase in the number of microglia by 50-55% in the hippocampus compared to the WT. The hippocampal microglia cell density in Tau58/2 mice was 30-33% higher than WT. The glial morphological analysis showed a different ramification and microglial cell body size in the GFAP-IL6 and GFAP-IL6XTau58/2 compared to WT and Tau58/2 in adult mice.

Poster

Sulfation pathways in neurodevelopment

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Sulfate is an important nutrient for healthy fetal growth and development. The content of sulfate in tissue is tightly controlled by 91 sulfate biology genes (also referred to as sulfation pathways) that are highly conserved between humans and animals. Adverse neurodevelopment is one of the most prominent features linked to disruptions of sulfation pathways. Despite the physiological importance of sulfate being documented over the past decades, the specific location/timing of sulfation pathways in the developing brain is not fully understood. To address this knowledge gap, this study investigated the spatial and temporal mRNA expression profiles of sulfate biology genes in the developing human brain.

Human brain mRNA expression levels of all known sulfate biology genes were curated from the GeneCards database (www.genecards.org/) which is a combination of data from The Genotype-Tissue Expression (GTEx), Illumina Body Map, Microarray BioGPS and Serial Analysis of Gene Expression (SAGE) databases. Spatial and temporal mRNA expression profiles of the most abundantly brain-expressed sulfate biology genes were curated from BranSpan (www.brainspan.org/) and plotted in GraphPad Prism version 9.

A total of 67 sulfate biology genes were abundantly expressed in human brain, including: 7 sulfate transporters; 2 PAPS (sulfate donor) synthetases and 2 PAPS transporters; 7 enzymes in the pathway of sulfate generation; 34 sulfotransferases; 13 sulfatases and 2 sulfatase modifying factors. Most of these genes were expressed at steady state throughout most regions of the brain between 8-37 weeks post-conception (wpc), whereas certain genes including the SLC13A4 sulfate transporter, showed a tissue-specific (including frontal and parietal cortices, and hippocampus) increased expression level between 12-20 wpc when sulfate-dependent processes such as neuronal migration and neuronal/glial proliferation occurs.

This study curated the expression profiles of sulfate biology genes in the developing human brain. These findings provide information for future investigations of the sulfation pathways associated with neurodevelopment.

Poster

Turnover dynamics of CNS border-associated macrophages in adult mice

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Border-associated macrophages (BAMs), including meningeal, perivascular and choroid plexus macrophages, play essential roles in neuroimmune surveillance and responses in homeostasis and neuropathology. However, compared with microglia, the innate parenchymal macrophages in the central nervous system (CNS), the ontogeny and functions of BAMs in health and disease remain relatively unknown. This project focuses on the turnover of the macrophages by circulating monocyte-derived immune cells at the CNS border. We adopted a transgenic model, SclCreERT2 x R26-ZsGreen mice, by which only bone marrow-derived BAMs rather than resident BAMs will be irreversibly marked when the mice are treated with tamoxifen. It prevents invasive surgeries like whole-body irradiation followed by Bone marrow transplantation, inducing strong immune responses. Using this method, we quantitatively analysed the peripheral monocyte-based BAM turnover of adult mice under the homeostatic and inflammatory conditions.

Poster

Tale of two anti-N-methyl-D-aspartate receptor (NMDAR) antibody-positive cases – Is it Autoimmune Encephalitis?

Dr Deepti Yagnik, A/P Kevin D O'Conor

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Case 1: A 53-year-old lady with three-year history of refractory depression and delusional disorder demonstrated serum anti-N-methyl-D-aspartate receptor (NMDAr) positive. She was on venlafaxine and olanzapine depot. The neurological examination was unremarkable except for blunted affect and delusional thinking. Electroencephalogram (EEG), magnetic resonance imaging (MRI) brain and pelvis, computed tomography (CT) chest, and positron emission tomography CT whole body and brain were normal. Cerebrospinal fluid (CSF) examination was bland with negative oligoclonal bands and +1 anti-NMDAR antibody. She was trialled with 5-day intravenous methylprednisolone (1gram) and immunoglobulins (IVIg). The IIF was negative for NMDAr.

Case2: A 39-year-old lady relocated interstate to WA in 2023 with an established diagnosis of NMDAR encephalitis in 2010, initially presenting with subacute behavioural changes and seizures. EEG demonstrated generalised slowing with no epileptiform abnormalities.CSF revealed pleocytosis (41 cells), positive oligoclonal bands with positive NMDArAbs in serum and CSF. She responded well to prednisolone, IVIg, antiseizure medications (phenytoin, valproate, and clonazepam), quetiapine and haloperidol. The pelvic imaging was negative for ovarian teratoma. She had a presumed relapse in 2014, presenting with isolated behavioural changes without recurrence of seizures. She was commenced on 6 monthly IV Rituximab (500mg) for serum NMDAr positivity and continued until January 2023, prior to our review. She was B cell deplete and serum NMDArAb negative in April 2023. She currently suffers from a complex post-traumatic stress disorder but has remained seizure-free. Repeat CSF NMDArAb and cerebral PET was negative. She was eventually taken off Rituximab.

Discussion: There are case reports for isolated psychiatric manifestations in NMDAR-related AE, although, most patients develop autonomic

dysfunction, seizures, or movement disorders. There was no associated inflammation on the MRI brain or PET scan, raising questions about AE diagnosis. It is important to cautiously consider clinical and paraclinical parameters when diagnosing NMDArAE.

Poster

Probable multifocal central nervous system graft-versus-host disease (CNS GvHD) with concurrent cerebellitis following allogenic stem cell transplant for T-cell leukemia

Dr Deepti Yagnik, A/P Kevin D O'Conor

Concord Repatriation Hospital

Case Report: 23-year-old dextral man was referred to Neuroimmunology clinic following a subacute onset asymmetric central cerebellar syndrome over preceding 4 months. He was diagnosed with T-cell acute lymphoid leukemia in August 2022 after presenting with confusion and hyperviscosity syndrome. He underwent leukapheresis, induction with Daunorubicin and Vincristine followed by consolidation cytarabine, cyclophosphamide, mercaptopurine and methotrexate. He had myeloablative chemotherapy followed by matched unrelated allogenic stem cell transplant in March 2023. He was initially treated with rituximab 700 mg intravenously weekly for a month, intrathecal hydrocortisone twice weekly, intravenous immunoglobulins induction followed by maintenance 4 weekly, intravenous methylprednisolone 1g two doses followed by oral prednisolone with a slow wean to 12.5 mg daily. He was also on cyclosporin 300mg BD and ruxolitinib 10mg bd.
He had pan-cerebellar signs with superimposed long tract signs on examination. A magnetic resonance imaging (MRI) of the brain with gadolinium demonstrated bilateral leukoencephalopathic process with tract involvement. An MRI spine was unremarkable. A cerebral FDG PET is pending. Serum anti-GAD, VGKC, anti MOG and JCV serology were negative. Cerebrospinal fluid (CSF) examination was non-inflammatory, CSF viral PCR including EBV, JCV, herpes simplex and antineuronal antibodies were negative. The patient was diagnosed with probable multifocal CNS GvHD with concurrent cerebellitis. Ciclosporin was recommended to be increased to 6mg/kg and an option of mesenchymal stem cell therapy was discussed.

Discussion: CNS GvHD following allogenic stem cell transplant is a rare condition. The management of CNS GvHD is unclear. The treatment usually involves steroids and in resistant cases alemtuzumab, cyclophosphamide and mesenchymal stem cells.