Mechanisms and Long term Consequences of Neuroinflammatory Disease

Summary of the 25th Nikolas Symposium, Athens, Greece May 14-17, 2015

Dedicated to the memories of Drs Robert Arceci and Marian Malone

The 25th Nikolas symposium was marked by mixed emotions. The meeting drew in experts with a wide range of backgrounds from paediatric neuro-imaging to adult neuro-oncology who reviewed many exciting new areas of progress since the theme of neuroinflammation was last discussed in 2010. However, shortly afterwards, news arrived of the tragic and untimely death of Dr Robert Arceci, a man whose charisma and scholarship had touched everyone. He is greatly missed by all and this report joins a long list of honours and commemorations that have been written in praise of his life and work. Sadly too, Dr Marian Malone, an inspiring and insightful pathologist who had led the discussions at many symposia, also died in 2015.

The meeting was opened by **Dr Robert Arceci** (Phoenix, USA) and **Dr Carlos Rodriguez-Galindo** (Boston, US now Memphis, US) who introduced participants to the clinical features and treatment of Langerhans cell histiocytosis (LCH; Allen et al., 2015). Historically LCH was known as Histiocytosis X, a unification of three syndromes: eosinophilic granuloma of bone, Hand-Schuller-Christian Disease (lytic skull lesions, diabetes insipidus and exophthalmos) and Letterer-Siwe Disease (high risk multi-system disease). Tissues of the head are affected in multiple different ways including cradle cap rash of the scalp, lytic granulomata of the vault of the skull, 'dancing teeth' due to erosion of the alveolar bone and parenchymal CNS lesions. The most commonly affected site in the brain is the pituitary stalk where inflammation and thickening ablates the posterior pituitary 'bright spot' on T2-weighted MRI and leads to diabetes insipidus. LCH also targets the hypothalamus and cerebellum presenting with a range of problems from tumour-like masses to late degenerative changes. Previous research suggested that involvement of the cranio-facial bones and base of skull increased the risk of degenerative CNS-LCH by three-fold. A question remains about the risk of CNS degeneration after isolated involvement of the pituitary. Dr Rodriguez-Galindo outlined the stratification of the current LCH IV international trial in which patients with CNS-LCH receive dedicated therapy. He ended by saying that CNS LCH remains one of the most significant unsolved challenges in terms of pathogenesis and morbidity, although molecular diagnostics and new agents offer considerable promise.

In Session II, **Dr Jennifer Picarsic** (Pittsburgh, US) summarized the pathology and diagnostic pitfalls of LCH. S100, CD1a and Langerin are mainstays of the routine diagnostic panel. Antibody VE1 recognising BRAF V600E and staining for phospho-ERK are newer tests that specifically detect aberration of the MAP kinase pathway driving neoplasia. The three critical diagnostic criteria remain: cytomorphology, immunophenotype and pattern of tissue involvement. However, difficulties can occur in older lesions where xanthomatous or fibrous infiltrates predominate and LCH cells are scarce, or in non-LCH conditions where CD1a+ dendritic cells (DCs) may be abundant such as Hodgkin's lymphoma, chronic dermatitis and dermatopathic lymphadenopathy. In the CNS, Dr Picarsic described several different groups of lesions: mass lesions; white matter changes; neurodegenerative disease; and atrophy. Although mass lesions are frequently biopsied and often display characteristic pathology, the other groups occur secondarily and are rarely sampled. The seminal studies on LCH CNS pathology, published by Nicole Grois and colleagues (Grois et al., 2010), show infiltration by T cells, macrophages and elements of neurodegeneration (loss of Purkinjie fibres; gliosis) with sparse or absent LCH cells.

Dr Hugh Perry (Southampton, UK) presented an overview of neuroinflammation. He commented that damage, degeneration and dysfunction are linked but not always all present in CNS inflammation. He emphasized the benefits gained through the use of new technology to quantify symptoms such as tremor, gait disturbance, ataxia, behavioural disturbance and cognitive impairment. Dr Perry distinguished between neuroinflammation and neurodegeneration. Distinct populations of macrophages are involved in each process. In a neuroinflammatory disease like multiple sclerosis (MS), there is a mixed perivascular infiltrate of T cells, macrophages and DCs. In neurodegenerative diseases, such as Alzheimer's disease (AD), protein misfolding is associated with prominent microglial activation without obvious inflammatory cell recruitment (Perry and Holmes, 2014). The processes can become connected because repeated inflammatory insult, as occurs in relapsing-remitting MS, leads to axonal damage and neurodegeneration (Heneka et al., 2015). GWAS studies in AD identify risk loci encoding genes that are expressed in microglia, suggesting that they play an active role in pathology. Activated microglia have a 'hybrid' phenotype with anti-inflammatory TGFβ, MCSF, IL-34 and pro-inflammatory MCP-1, CCL2, IL-1, IL-6. Their proliferation rate increases above the basal rate of 0.5% in response to M-CSF and IL-34 (Gomez-Nicola and Perry, 2016). Inhibition of the M-CSF axis by kinase inhibitor GW2580 delays the onset of developmental symptoms and death in animal prion models but it is not proven that this is due the suppression of microglia as opposed to other macrophages population in the animal (Olmos-Alonso et al., 2016). Dr Perry went on to explore the role of systemic inflammatory responses in promoting neurodegeneration, illustrated by systemic infection in prion models of neurodegeneration and supported by clinical observations in which acute infection is documented to accelerate cognitive decline. Immune to brain communication can occur in several ways: through vagal sensory fibres; at sites where the blood brain barrier is open; and across the vascular endothelium. The posterior pituitary is especially vulnerable because oxytocin containing neurons pass outside the brain parenchyma to be trimmed by perivascular macrophages. This unique relationship between neurons and macrophages creates the potential for activated macrophages to infiltrate retrogradely from the perivascular space into the posterior pituitary. The cerebellum is frequently involved in paraneoplastic processes and is a major target of LCH degenerative disease. This suggests a linked aetiology, although the reason for cerebellar tropism in either condition is unknown. Dr Perry speculated that there may less resilience to inflammatory insults that might trigger immunogenicity of cerebellar antigens owing to the lower density of resident microglia compared with other areas of the brain. He noted that few paraneoplastic degenerative conditions exist in which humoral immunity to a cognate antigen has been defined and that even greater challenges are presented by a cell-mediated immune processes driven by T cell epitopes. LCH is not alone as a CNS inflammatory disease without a clearly defined aetiology and major barriers exist in understanding how to arrest or even reverse any neurodegenerative disorder. In relation to systemic inflammation promoting AD, peripheral production of TNF may play a role and Etanercept is being investigated as a potential therapy.

Dr Florent Ginhoux (SIgN, Singapore) gave a talk on the origin of DCs and macrophages and how this could influence CNS inflammation. He began by reviewing the ontogeny of the mononuclear phagocyte system and the differences between the homeostasis of DCs and macrophages (Ginhoux and Jung, 2014). The contribution of circulating monocytes to macrophage populations has been over-emphasised through animal models using predominantly inflammatory conditions and poorly defined BM-derived monocyte fractions that contain additional more primitive myeloid precursors. Although skin and gut macrophages arise continuously from monocytes, as proposed in the original definition of the mononuclear

phagocyte system, liver Kupffer cells, lung alveolar macrophages and microglia of the brain are maintained independently. Dr Ginhoux's previous work has shown that microglia arise from yolk sac progenitors early in development while other macrophage populations arise later from foetal liver monocytes (Ginhoux et al., 2010). Both yolk sac and foetal liver monocytes are ultimately derived from primitive erythro-myeloid precursors that originate in the yolk sac, prior to the formation of definitive haematopoietic stem cells in the foetal liver (Hoeffel et al., 2015). Microglial precursors populating the developing brain form hotspots of expansion in certain sites for example in proximity to nascent dopaminergic neurons. Depletion of microglia can be achieved experimentally by intra-uterine injection of anti-MCSFR antibodies resulting in alterations in brain architecture such as transient axonal over-growth and repositioning of neocortical interneurons (Waisman et al., 2015). The developing microglial network is also influenced by the microbiome and fewer more activated microglia are found under germ-free conditions. Surprisingly to scientists who have studied the independent homeostasis of microglia, depletion by anti-MCSFR antibodies resulted in post-natal repopulation by foetal liver derived monocytes. This implies that conditions exist where bone marrow derived cells may be recruited to form new microglia.

Session II was concluded by **Richard Ransohoff** (Cambridge, US) who also spoke about myeloid cells in the CNS. He continued the theme of development and recapitulated the story that microglia have a primitive haematopoietic origin (Ransohoff and El Khoury, 2016). Microglia have dual roles in development and inflammation and there are many examples of inflammation recapitulating ontogeny through the exploitation of common molecular mechanisms, sometimes clearly linked to deleterious effects of inflammation. For example, the development of retinal projections to the lateral geniculate nucleus depends upon the local production of complement C1q to direct microglia to perform phagocytic synaptic pruning. This is a non-inflammatory remodelling process during development but reactivation of complement in the adult brain by inflammation reawakens microglial phagocytosis causing neuronal damage. Dr Ransahoff presented a 'helicopter parent' view of the interaction between microglia and neurons, in which the microglial 'parent' is continually interrogating the neuron 'child' and rapidly intervenes if there is no affirmative response. Immune signalling molecules CX3CR1/L1, CD200 and TREM2 regulate microglial activation in the post-natal brain. CX3CR1 knockout mice have impaired neuronal connectivity and social behaviour and are more susceptible to neurodegeneration in the MTPT model of Parkinson's disease and to the growth of gliomas (Feng et al., 2015). In models of neurodegeneration, tau-mediated pathology is exacerbated but amyloid deposition is reduced (Lee et al., 2014). Genome-wide association studies (GWAS) identified TREM2, a receptor expressed both by resident microglia and recruited monocyte-derived macrophages, as a major risk factor for late-onset AD. TREM2+ cells accumulate at plaques and TREM2 knockout mice are protected from neurodegeneration, however it is not clear if pathology is mediated by microglia, recruited macrophages or both (Jay et al., 2015). The question of resident versus recruited macrophages pervades many pathological states. Bone marrow derived macrophages are found in the meninges, the perivascular spaces and at CSF drainage through the cribriform plate where they survey memory CD4+ T cells leaving the CSF. Generally, bone marrow-derived cells accumulate in the brain parenchyma with increasing age. Experiments have been performed to dissociate microglia and recruited monocytes using CX3CR1 GFP and CCR2 RFP co-labels, respectively. Confocal fluorescence image stacks registered with EM image stacks obtained after an inflammatory insult, showed that recruited monocytes but not microglia actively stripped myelin sheaths, beginning from the nodes of Ranvier (Yamasaki et al., 2014). Timed analysis at different stages in the evolution of inflammation showed two cell lineages distinguishable by origin and never sharing a common phenotype (Dal-Secco et al., 2015). Current work was focused at dissecting the time-dependent regulation of gene expression in microglia and recruited cells in wild type and knockout mice, to

achieve further insights into the response of the brain to inflammation (Crotti and Ransohoff, 2016).

In session III, **Dr David Edwards** (London, UK) gave a review of imaging techniques in CNS development and disease. He began by summarising the human connectome project in which investigators are seeking to map all the connections of the human brain. At the EM level this is a huge undertaking and requires post-mortem specimens. At the macroscopic level it is possible to map white matter connections with a 'connectogram' in which anisotropy in water molecule diffusion is used to map nerve fibre tracts. Anisotropy increases with post-natal age consistent with maturation of dendritic arborisation (Tusor et al., 2014). Spatial analysis indicates that arborisation progresses in a wave across the cortex beginning with the primary sensory areas. Another technique, functional connectivity MRI (fcMRI) monitors coordinated brain activity, from which anatomical connectivity can also be inferred (Toulmin et al., 2015). Network theory is then applied to develop a matrix of connectivity between 83 different brain regions that can be monitored over time to study the developing brain (Ball et al., 2014). Specific parameters, such as the development of thalamo-cortico connectivity within the first two years, are able to predict cognitive performance later in life (Ball et al., 2012). These investigations reveal disruptions of connectivity and brain maturation with inflammation that can be correlated with % CD45RA+ cells present in biopsy or autopsy sections. Gene expression differences, many relating to lipid metabolism and inflammatory mediators, are beginning to be correlated with cortical development and connectivity using tools such as the Allen Brain Atlas (www.brainspan.org) .

One of the ultimate aims of the research described by Dr Edwards is to be able to use non-invasive imaging to study neuropathology at the molecular level. This theme was developed by the next speaker **Dr Michelle James** (Stanford, US) who described novel agents to facilitate the molecular imaging of inflammatory disease with positron-emission tomography (PET). PET has excellent sensitivity and depth of penetration but poor spatial resolution and hence is usually combined with CT or MRI. In addition to Flurodeoxyglucose (FDG), newer agents targeting specific cells or enzymes, such as ^{64}Cu-rituximab for B cells or ^{11}C-deuterium-L-deprenyl (DED) for monoamine oxidase, are being used to map inflammatory responses. There is great interest in studying microglial activation owing to its perceived importance in neurodegeneration and AD. The mitochondrial translocator protein (TSPO) is a marker of microglial activation targeted by promising new compounds such as the fluorine adduct ^{18}F[GE-180]. A particular translational goal is to be able to observe a microglial response to drug trials in AD.

Dr Jenny Ting (North Carolina, US) delivered a talk on molecular approaches to inflammation with a focus on multiple sclerosis. Her group characterised the NOD-like receptors that are critical components of inflammasomes and that are mutated in periodic fever syndromes (Freeman and Ting, 2016). NLRP3 is activated by a number of chemicals including uric acid, ATP, cholesterol, amyloid and other mediators that may be involved in neuroinflammation. Dr Ting described that both experimental allergic encephalomyelitis (EAE) and copper chelation (cuprizone) models of demyelination are NLRP3-dependent. Intriguingly, mutation of the flagellin sensor NLRC4, that had no known function in chemical inflammation and was intended as a negative control, also reduced disease severity. This led to the discovery that NLRC4 associates with NLRP3 by mass spectrosocopy and super-resolution microscopy and is likely to be a co-factor for NLRP3-mediated inflammation. Dr Ting then turned her focus to NLRX1, a NOD-like receptor that represses immune responses through inhibition of the MAVS-RIG1 interaction necessary for recognition of viral nucleic acid. NLRX1 knockout mice experience exacerbated EAE and have increased levels of IL1 and IL6 in a number of challenge models (Eitas et al., 2014). Inflammation-induced neoplasia is also enhanced in association with increased levels of pSTAT3 in stromal cells. Together these studies illustrate that molecular

activation of the inflammatory response links infectious agents, chemical mediators and neoplastic transformation.

Dr Josep Dalmau (Barcelona, Spain) spoke on paraneoplastic and autoimmune encephalitis at the start of session IV. He began by saying that encephalitis causes 20,000 admissions per year to US hospitals. One of the most striking examples of a paraneoplastic encephalitis is mediated by anti-NMDA receptor antibodies, associated with ovarian teratoma or HSV-1 encephalitis (Lancaster and Dalmau, 2012). Several hundred patients have been described worldwide (Titulaer et al., 2013). The syndrome is preceded by headache and fever then develops rapidly into catatonia, psychosis, memory loss, speech defects orofacial dyskinesia. Patients may become comatose and require ventilation. Imaging and EEG are often normal but in the majority of cases (85%) there is lymphocytic pleiocytosis with increased protein and oligoclonal bands in the CSF. Plasma cells may be seen in the meninges at autopsy in the minority who perish. For 80% of patients, a slow recovery with total amnesia ensues. Treatment requires plasma exchange, rituximab and cyclophosphamide and relapse is rare if a teratoma, which is the trigger in 80%of patients, has been removed. Antibodies to residues 368-9 in the ligand binding domain of the NMDA receptor are found in higher titre in the CSF than the serum and decorate the hippocampi in brain sections. The NMDA receptor is rapidly cross-linked and internalised resulting in loss of signalling that recapitulates pharmacologic or genetic ablation of the NMDA receptor. Patient CSF introduced into the ventricles of mice causes impairment of memory and development of anhedonia, in association with loss of NMDA receptor expression. The effect could be overcome with ephrin receptor stimulation owing to the fact that ephrin receptor and NMDA receptor associate at the plasma membrane. Since the description of NMDA receptor antibodies, more than ten pathogenic anti-receptor antibodies have been described (Leypoldt et al., 2015). HSV-1 is a clear trigger causing post-encephalitis choreo-athetosis to a degree in up to 25% of patients according to prospective studies (Armangue et al., 2015). Clinical signs may be subtle and Dr Dalmau suggested that careful examination of CSF and plasma from LCH patients with neurodegenerative disease against brain slice target antigens might disclose low titre or low affinity anti-neuronal antibodies. He commented that humoral paraneoplastic disease remains much more amenable to treatment compared with T cell mediated phenomena such as those driven by anti Ma2 responses that may never recover (Lancaster and Dalmau, 2012). A concern is that the difficulty of treating LCH neurodegeneration could be consistent with the latter process.

Dr Ann Yeh (Toronto, Canada) gave insights from her experience of multiple sclerosis and neuroinflammation in children. She began by describing a number of cases to illustrate that the depth of symptoms and imaging abnormalities at presentation were both poorly correlated with the extent of spontaneous recovery and long term disability. From these considerations, it is difficult to formulate treatment algorithms that will not aggressively over-treat a proportion of patients. LCH is a more aggressive example and other abnormalities that mimic its presentation may resolve spontaneously. Looking at the epidemiology, Dr Yeh noted that the incidence of first-time demyelination is about 1 per 100,000 in children, considerably more common than CNS-LCH. Only 20% of children have MS; the other 80% have an acute demyelinating encephalomyelitis (ADEM) that has an identifiable precipitant, in about half of cases, such as genetic, vasculitic or autoimmune. Although an inexact science, some guarded predictions may be derived from imaging; for example, the presence of demyelination on MRI beyond the age of 12 is associated with a 60% risk of MS. Whatever the outcome, it is clear from the paediatric MS series that diffuse parenchymal injury and reduced brain volume are pervasive and pernicious problems. Neurocognitive deficits frequently arise even in those children that are superficially normal. Visual motor processing, attention and memory are impaired on rigorous neuropsychological testing and depression and fatigue are more common than in healthy peers.

The important message for LCH is that all patients should undergo neuropsychological testing even when cognition is grossly normal. Echoing the sentiment of several previous speakers, it was concluded that one of the key starting points for understanding neurological disease in LCH is a careful and thorough clinical evaluation.

The theme of demyelinating disease in children was continued by **Dr Mark Tardieu** (Paris, France) with a talk on the role of environmental factors in paediatric multiple sclerosis. He reminded the audience that the main difficulty was to predict the difference between a single acute event and the onset of chronic disease. He summarised known risk factors: there is an 8% chance that children with MS have an affected first degree relative (e.g. due to inheritance of HLA-DRB1); prior EBV infection increases the risk by nearly 3 fold; childhood VZV infection decreases the risk; low sun exposure and vitamin D increase the risk and exposure to smoking approximately doubles the risk. It is unknown how many of these environmental factors influence the immune system. One strand of recent research highlights how T cells licensed in distant organs can recirculate to the CNS where they are primed by perivascular macrophages (Odoardi et al., 2012). This would allow exposure to a range of environmental stimuli such as steroid hormones and microbiota. Adhesion molecules such as Ninj1 and VLA-4 are involved lymphocyte trafficking and a range of new therapeutics is aimed at ablating lymphocytes or preventing their recirculation (alemtuzuamb, natalizumab, fingolimod and lefluonamide). Dr Tardieu recommended rapid and intensive induction therapy in new patients with corticosteroids, plasmapheresis and interferon β or glatiramer acetate. Patients who relapse are treated with alemtuzumab or natalizumab, the most promising second line agents. In line with previous speakers, Dr Tardieu recommended regular close assessment of imaging and neurocognitive function even in patients who have responded well to therapy (Banwell et al., 2011).

Session IV was concluded by **Dr AnnaCarin Horne** (Stockholm, Sweden, Jon Pritchard Fellow) who looked to neuroinflammation in HLH to provide lessons for LCH. Neurological complications in HLH occur frequently: 32% incidence of neurology at diagnosis; 37% chance of abnormal MRI; 56% chance of abnormal CSF; and 37% risk of long term sequelae, usually cognitive impairment. Neurological involvement in HLH remains an independent risk factor for death. Neuropathology in HLH is thought to arise from the meningeal vessels. HMGB1 is released and could potentially mediate neurotoxicity, although few data are available to support this experimentally. Studies with Luminex panels are underway to compare the CSF from patients with encephalitis and HLH.

Dr Staci Bilbo (Carolina, US) opened the final session (seesion V) with a talk entitled: 'Neural development and cognition throughout the lifespan: a critical role for the immune system'. Her approach was to examine developmental programming in the CNS and the effect of immune mediators on neural development in animal models of behaviour. As noted earlier in the symposium, classical immune mediators such as IL-1β also have developmentally regulated expression patterns in the CNS, in the absence of inflammation. Dr Bilbo talked about the origin of microglia, pre-empted by Hortega, a student of Ramon y Cajal, who referred to them as 'invaders' from another tissue. The development of microglia follows a discrete route from the corpus callosum, the morphology of cells passing through migratory amoeboid states to 'reactive-looking' appearances at birth and finally reaching a mature arborized form in early life (Bilbo and Schwarz, 2012). The reactive appearance at birth is entirely normal and does not reflect infection. There are distinct patterns of microglial gene expression from prenatal to post-natal life that are region-specific (Schwarz et al., 2012). For example, the expression of IL-1β peaks at day of birth in the cortex but one week later in the cerebellum. (Bilbo and Schwarz, 2009). Although viral infection is often mooted as a potential neuromodulatory event, Dr Bilbo

argued that neonatal sepsis was a more common and severe insult to the neonate. She described a model in which neonatal sepsis was followed by a second exposure to LPS that resulted in impairment of a memory task. The stimulus did not induce peripheral cytokine activation but did induce microglial IL-1β and had specific effects upon hippocampal gene expression that correlated with memory impairment. Both neonatal sepsis (within 30 days) and LPS challenge were required for impairment of the memory task and intraventricular infusion of caspase-1 inhibitor or the quiescence-inducing antibiotic minocycline could restore performance. Sepsi did not induce obvious functional changes in microglia and almost all activation markers normalised prior to the LPS challenge (with the exception of a larger cell body). Sepsis alone, without subsequent LPS challenge, did not impair the specific memory task but resulted in changes in social interaction tasks, making animals more wary and anxious. Conversely, LPS challenge alone began to impair memory performance in aged rats in proportion with the increase in microglial activation observed with ageing. In seeking to understand the ability of sepsis to modulate microglial function, ongoing work included studies of epigenetics, expression levels of CX3CR1, CD200 and other measures of microglial reactivity. In addition to neonatal sepsis, it was noted that the health of the mother may have protective or deleterious effects upon resident microglia and ensuing neural development.

Dr Eli Diamond (New York, US) brought the focus back to patients with histiocytic disease in the concluding presentation. He presented data on Erdheim Chester Disease (ECD), including recent work describing a common aetiology between ECD and LCH in the genetic dysregulation of MAPkinase signalling (Diamond et al., 2016). ECD is a multi-system disease that tends to be consistently distributed within an individual. Chronic inflammatory (B-type symptoms) are prominent with raised cytokines and inflammatory markers. Diagnosis is often delayed owing to the protean nature of the disorder and radiological criteria are usually more sensitive and specific than tissue biopsy in many cases. Typical pathology with foam cells is found most frequently but not invariably in bone and skin lesions. CNS lesions can be very pleiomorphic with mixed or predominantly lymphoid infiltrates. Staining with anti-pERK antibodies can be very useful in mixed or biphasic lesions where histopathology is atypical. Both ECD and ECD/LCH show rapid responses to treatment with targeted kinase inhibitors and metabolic responses are generally much quicker than anatomical resolution. Infiltrative ECD typically affects that pituitary, brainstem, cerebellum, dura, and may involve the spinal cord. Degenerative ECD is more tropic for the cerebellum and brain stem causing ataxia, oculomotor abnormalities and dysphagia. Although some sites like the dura are characteristic of ECD, there is overlap with the patterns seen in LCH, especially in the sites affected by degenerative disease. Cognitive dysfunction is suggested by the observation that patients with good physical fitness are frequently unemployed. Dr Diamond described an MRI study in 14 previously chemo-naïve ECD patients without overt CNS involvement using high resolution volumetric T1 MRI and the FreeSurfer platform. Cortical thickness was significantly and globally reduced in ECD brains by a surprising degree compared with age-adjusted normal brain scans. Subcortical grey matter was also significantly reduced in volume. Indirect explanations such as an association with cancer, systemic inflammation or nutritional deficiencies were considered. CSF analysis was uniformly bland but many patients had severe nutritional deficits and hypogonadism. Thus, although ECD is considered to cause primary CNS pathology, secondary effects especially nutritional status, merit serious consideration for clinical intervention. The image analysis of ECD patients amply demonstrated the need to monitor cortical parameters in LCH patients in parallel with detailed clinical evaluations.

The symposium was summarized by **Dr Hugh Perry** and **Dr Peter Beverley**. Many useful animal models of inflammation and degeneration were discussed that have now begun to yield mechanistic insights. Microglia and recruited myeloid cells remain central to the process of

neuronal damage. From the clinical perspective, MS and AD offer a wealth of experience in imaging, pathology and therapeutic trials that are relevant to LCH. Subclinical neurodegenerative disease may be much more prevalent than previously suspected and more detailed clinical and radiological examination of all LCH patients is warranted. One view of the late effect of LCH is that it is an inevitable consequence of CNS involvement, similar to that seen in ECD. Thus, the apparently unpredictable occurrence of LCH neurodegeneration may reflect inadequate evaluation of CNS disease during the initial presentation, rather than an inherently stochastic or indirect process. Although much remains to be learned, fruitful avenues of exploration are now clearly identified.

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