Leonard Wolfson Experimental Neurology Centre
Annual Symposium 2017

Inflammation and immunology: the solution to neurodegenerative disease?

Monday 20 March, 2017
Institute of Child Health
Hosted by: LWENC, Institute of Neurology, UCL
Programme

10.00-10.30 Networking session with coffee and tea

10.30-10.50 Welcome
   John Hardy

Session 1 chaired by Tammmaryn Lashley

10.50-11.20 Innate Immune Activation in Alzheimer's disease - a potential therapeutic target?
   Michael Heneka

11.20-11.40 Linking GWAS and inflammation in AD - what we now know about microglial function
   Jenny Pocock

11.40-12.00 Lessons for neurodegeneration from multiple sclerosis?
   Ken Smith

12.00-1.30 Lunch and Poster Session

Session 2 chaired by Jonathan Rohrer

1.30-1.50 Autoimmune synaptic disorders and implications for treating inflammation in Alzheimer's Disease
   Mike Zandi

1.50-2.10 The TREM2 story in dementia
   Rita Guerreiro

2.10-2.30 The progranulin story and the role of inflammation in FTD
   Ione Woollacott

2.30-3.15 Refreshments and Poster Session

Keynote lectures chaired by John Hardy

3.15-4.00 Keynote: Microglial dynamics in health and disease
   Diego Gomez-Nicola

4.00-4.45 Keynote: TBA
   Roger Nitsch

4.45-5.00 Closing Remarks & Poster Prize

5.00-6.00 Drinks reception

N.B. This event is being filmed by UCL Media and will be streamed live and the recording will be made available on a UCL approved media outlet.
Induced Pluripotent Stem Cell-Derived Macrophages as a Model to Study the Role of TREM2 in Alzheimer’s Disease

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Background:
Heterozygous variants in the immune receptor gene Triggering Receptor Expressed on Myeloid Cells (TREM2) were recently found to be associated with Alzheimer’s disease (AD). Homozygous mutations in the TREM2 gene cause polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), also known as Nasu-Hakola disease, an early-onset dementia characterized by behavioural change and cognitive decline, with or without pathological bone fractures. How TREM2 contributes to AD pathogenesis is poorly understood.

Methods:
To study the role of TREM2 in AD pathogenesis, we generated human induced pluripotent stem cell (iPSC)-derived macrophages (referred to as iPSC-MΦ) from two patients with Nasu Hakola dementia caused by a homozygous T66M or homozygous W50C TREM2 mutation, two carriers of a heterozygous T66M TREM2 mutation, and three controls. iPSC-MΦ were analyzed using immunocytochemistry, western blotting, fluorescence-activated cell sorting (FACS), and enzyme-linked immunosorbent assay (ELISA).

Results:
Human iPSC-MΦ expressed a variety of macrophage markers as well as the tissue resident macrophage markers CSF1R and TREM2, the latter being secreted into the extracellular space. TREM2 mRNA, protein levels and glycosylation were reduced in T66M heterozygous, T66M homozygous and W50C lines. TREM2 secretion was reduced in both T66M heterozygous mutant lines and virtually abolished in T66M homozygous and W50C homozygous iPSC-MΦ. Secretion of the cytokine tumor necrosis factor alpha (TNF-α) but not interleukin 6 (IL-6) in response to lipopolysaccharide (LPS) was increased. Phagocytosis of E.coli bacteria, but not zymosan particles was impaired in TREM2 homozygous but not heterozygous iPSC-MΦ. Characterisation of these cells allows us to conclude that they are a good model in which to study the ramifications of TREM2 mutations for immune cell dysfunction in dementia. Furthermore our findings demonstrate that TREM2 homozygous (but not heterozygous) mutations cause an increase in TNF-α release in response to LPS and affect phagocytosis in a substrate-specific way.
TREM2 Coordinates Anti-inflammatory Responses and Phagocytosis in Primary Microglial Cultures


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Background:
Neuroinflammation is one of the major pathological features in Alzheimer’s disease (AD), and SNPs in immune genes like TREM2 and CD33 are found to be risk factors for AD by human GWAS studies. Here we use siRNA to knockdown Trem2 in primary microglia and investigate changes in microglial gene expression and function.

Methods:
Primary microglia, generated from 1-3 day old wild type mice, were transfected with Trem2 siRNA. Trem2 knockdown level and expression levels of immune genes were assessed by qRT-PCR. Phagocytosis was assayed using pHrodo E. coli bioparticles and analysed with fluorescence-activated cell sorting. Pro- or anti-inflammatory activation was induced by lipopolysaccharide (LPS) or interleukin-4 (IL4) treatment. All experiments in accordance with Animals (Scientific Procedures) Act 1986

Results:
At 72-hour after siRNA transfection, >70% knockdown of Trem2 was obtained in primary microglia at the mRNA level. Cd68, Csf1r, Igf1, Pi3kcg, and Spi1 (normalised to Aif1) were found to be significantly decreased after Trem2 knockdown. Phagocytosis was also significantly impaired. Expression of Trem2 was substantially depressed by LPS treatment, and not surprisingly Trem2 knockdown did not alter the up-regulation of pro-inflammatory genes (Tnf and Il1b) in response to LPS. However, with IL4 treatment Trem2 expression showed a significant time-dependent increase, and Trem2 knockdown significantly reduced the level of the up-regulation of genes like Arg1, Csf1r, and C1qa in response to IL4.

Conclusions:
Trem2 expression is largely down-regulated in primary microglia with LPS, suggesting pro-inflammatory stimuli depress TREM2-related functions of microglia. TREM2 is involved in microglial anti-inflammatory responses and maintaining normal phagocytic function.

Decreased expression/function of TREM2, due to loss-of-function variations in the gene or accumulated pro-inflammatory stimuli, may shift the gene expression profile and functional phenotype of microglia, which might contribute to AD progression.
Peptidylarginine Deiminases (PADs) – Novel Drug Targets for the Amelioration of Neurodegenerative Disease Progression - Modelled in Human iPSCs

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Background:
Progressive neurodegenerative diseases are often referred to as protein misfolding disorders, as common features are calcium dysregulation and accumulated misfolded protein aggregates that lead to fatal neuronal loss. Peptidylarginine deiminases (PADs) are a group of Ca²⁺-activated enzymes which cause irreversible post-translational conversions of protein bound arginines to citrullines in target proteins, changing their structure and function. Novel roles have been shown for PADs in acute CNS damage, where pharmacological PAD-inhibition resulted in significant dampening of neuroinflammatory microglial activation and neuroprotective effects in vivo. We have also shown a novel mechanistic role for PADs in the biogenesis of extracellular vesicle (EV) release, via deimination of cytoskeletal proteins and histones, which can be effectively targeted using PAD-inhibitors. Protein deimination and extracellular vesicle release are both Ca²⁺-mediated mechanisms which are increasingly associated to neurodegenerative diseases.

Methods:
We are currently using human induced pluripotent stem cell (iPSC) models to elucidate the involvement of PADs and post-translational protein deimination in neurodegenerative disease progression. We are testing neuronal cell lysates, generated from fibroblasts derived from patients carrying mutations for Parkinson’s disease (PD), frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) for expression of PAD isozymes, changes in deiminated target proteins and EV release. Results: We have found that protein deimination is increased in cell lines carrying certain FTD-ALS linked valosin-containing protein mutations (VCPR155C and VCPR191Q) and in the Parkinson Disease α-synuclein triplication. Specific targets identified include nuclear histone H3, which is involved in gene regulation and EV release.

Conclusion:
Human iPSC neurodegenerative disease models can be used to investigate PAD-mediated mechanisms involved in neurodegenerative pathologies and to test pharmacological PAD-inhibitors for intervention in neurodegenerative disease progression.
Assessment of Immune System Activation Status During the Course of Disease in Huntington’s Disease Mouse Model

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Background:
A number of neurodegenerative diseases, characterised by progressive and selective death of neurons in the CNS, are accompanied by activation of the peripheral immune system. In Huntington’s disease (HD), clinical and animal studies show elevated immune factors that are hallmarks of immune activity and the use of immunosuppressive regimens have shown beneficial effects in HD mice. These results suggest a contributory role of the immune system in HD pathology, with immune based interventions offering potential therapeutic strategy to disease.

Aim:
To assess peripheral and central nervous system (CNS) immune system activity in HD mouse model during disease to determine if / when peripheral immunomodulation will be relevant for HD treatment.

Methods:
Pre and post-symptomatic stages R6/2 mice were investigated for immune activity in the brain and periphery through the assessment of gene expression and protein levels of interleukin (IL)-1β, IL-6, IL-10, IL-17 and tumor necrosis factor (TNF)α cytokines. Gene and cell surface (flow cytometry) expression of monocyte and macrophage activation (CD40 and OX40l) and T cell activation (OX40 and CD25) markers were also measured.

Results:
At 14 weeks’ old (late-stage disease) cytokines and cell surface markers are elevated in several peripheral compartments as well as the brain. At 8 weeks (pre-symptomatic stage) however, immune activity is detectable only in the periphery.

Conclusion:
Immune activity in the periphery precedes immune activation in the CNS suggesting the peripheral immune system may promote activation of the CNS immune system during HD, possibly through the secretion of pro-inflammatory factors that cross the blood brain barrier. Additionally, myeloid immune cells in the CNS and periphery display differing activated phenotypes during late stage HD with microglial cells presenting a predominantly M1 phenotype while monocyte/macrophages show a M2 predominating phenotype.

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Investigation of amyloid and microglial pathology in sporadic, familial and TREM2 variant Alzheimer’s disease cases

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Background:
TREM2 was discovered as a genetic risk factor for late onset Alzheimer’s disease (AD) with a similar odds ratio to APOE. TREM2 is expressed on microglia and is thought to play a role in clearing amyloid plaques via phagocytic mechanisms. Phagocytic microglia have an amoeboid morphology whereas other microglia are more ramified in appearance. Previous studies suggest that TREM2- mice have less dense amyloid plaques than TREM2+ mice when crossed with amyloid producing mice (5xFAD). Here we investigate the amyloid and microglial pathology in sporadic, familial and TREM2 variant AD human post-mortem tissue.

Material and methods:
Eight µm sections from human frontal cortex, temporal cortex, hippocampus, putamen and midbrain were cut from sporadic (n=8), familial with both PSEN1 and APP variants (n=11) and TREM2 variant (n=4) AD cases. Immunohistochemistry was performed using Aβ, Iba1, CD68 and CR3-43 antibodies. Regions of interest were selected and ten random squares were generated from each case using a Python script. Area density analysis was performed for all antibodies. Cases were blinded and the number of diffuse and dense core amyloid plaques were counted. ImageJ was used to assess the circularity of the microglia.

Results:
The Aβ area density was reduced in TREM2 variant cases compared to sporadic and familial cases in the CA1 region of the hippocampus, putamen and the midbrain whereas levels were similar in frontal and temporal cortices. There were more diffuse plaques compared to dense core plaques in all regions. There was a higher density of Iba1+ and CD68+ microglia in TREM2 variant cases in the frontal cortex compared to sporadic AD cases with no TREM2 variant, whereas there were fewer CR3-43+ microglia.

Conclusions:
This data highlights that TREM2 variant AD cases do have a different pathological profile to sporadic and familial AD cases. A reduction in dense core plaques in some areas may suggest that TREM2 plays a role in amyloid plaque fibrillisation. Add to this the differences in microglial morphology between TREM2 cases and other AD cases and we can hypothesise that TREM2 alters microglial morphology which in turn may alter their response to amyloid deposition.
Genetic and proteomic profiling of sporadic and familial Alzheimer’s disease post-mortem brains

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Objectives:
The underlying pathogenesis of Alzheimer’s disease (AD) remains elusive. However recent observations indicate that neuroinflammation may play a role in the causative mechanism. Here we explore the gene expression profiles of 286 inflammatory/AD-related genes in post-mortem brains from sporadic AD and familial AD comparing to the proteomic profile in the same cases.

Methods:
RNA was extracted from the frontal cortex of sporadic AD (n=10), familial AD (n=7), and normal controls (n=6) using the Qiagen RNeasy kit. Samples were analysed using the Nanostring human inflammation panel including an additional 30 genes implicated in AD. Proteins were extracted and analysed using label-free mass spectrometry with a SYNAPT G2-Si High Definition machine with 2D fractionation.

Results:
Compared to controls, of the 286 genes analysed, 126 genes had statistically different expression levels (p<0.05) in sporadic AD and 93 genes in familial AD. A total of 4334 proteins were identified using mass spectrometry. Compared to controls, 163 proteins had statistically different expression levels in sporadic AD and 213 proteins in familial AD. Greater variation of up- and down- regulation was seen at the protein level compared to being predominantly up regulated at the gene level.

Conclusions:
Inflammatory gene expression differs between sporadic AD and familial AD indicating the two disease groups may not share a common inflammatory response pathway. However, alterations in the protein expression of some of these upregulated inflammatory genes suggests that there could be compensatory mechanisms occurring at the translational level.
Abnormal mitochondrial dynamics play a role in axonal degeneration in a model of inflammatory neuropathy

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The role of impaired mitochondrial trafficking is increasingly recognised in the pathogenesis of peripheral neuropathies, but their behaviour in vivo is very poorly understood. To study the effect of inflammation on mitochondrial function and transport, mitochondria were observed by time lapse confocal imaging in the exposed saphenous nerves of anaesthetised mice with experimental autoimmune neuritis (EAN). We found that, in comparison with naïve animals, the mitochondrial trafficking was increased in both EAN and adjuvant-only control animals. However, the number of mobile mitochondria was significantly lower in animals with EAN compared with adjuvant controls (p<0.001) or asymptomatic animals (p<0.001). Interestingly, at the onset of EAN, but not in matched asymptomatic or adjuvant-only control animals, we observed a number of small to medium diameter fibres (3.34+/-0.61µm) which contained focal accumulations of stationary mitochondria. The accumulations started abruptly at the proximal end, but ‘tailed off’ gradually distally, over several tens of microns. Mitochondrial movement was absent at this tail end, and further distally. The accumulated mitochondria were polarised, thus seemingly healthy. Time-matched, asymptomatic animals with EAN showed few, if any such accumulations, but we were able faithfully to reproduce these accumulations by laser damaging (photo-bleaching) the mitochondria in such axons. The damaged mitochondria became depolarised, fragmented and immobile, presumably depleting the energy supply of the affected portion of the axons. Interestingly, the more proximal mitochondria in these axons started to move towards the damaged region in significantly increased numbers than before photo-bleaching (p=0.007). Upon arriving they slowed or stopped moving, seemingly obstructed by the damaged mitochondria. The increase in proximal mitochondrial movement occurred in all axons, but it was only in small axons (2.7+/-0.45 µm) that the accumulations occurred. Thus in the larger axons alone the mitochondria passed unobstructed into the damaged field, repopulating it with healthy mitochondria. We suggest that failure of mitochondria to re-populate small axons may help to explain the selective loss of smaller axons in some peripheral neuropathies.
Adenosine deaminase (ADA) deficiency is a severe combined immunodeficiency (SCID) with a prevalence of approximately 1:200,000. Patients present early in life with severe lymphopenia and a failure to thrive. ADA deficiency is fatal if left untreated, and treatment options include enzyme replacement therapy (ERT), haematopoietic stem cell transplantation (HSCT) and gene therapy.

The immunological component is well elucidated - it is widely accepted that the accumulation of metabolic substrates, caused by deficiency of ADA, is lymphotoxic. However, expression of ADA is ubiquitous and patients exhibit a multi-organ pathology including neurological abnormalities both pre and post treatment. The current role of ADA in the brain is largely unknown; it is also unknown why current treatments do not effectively prevent the generation of and/or development of these abnormalities. Both in vitro and in vivo approaches are being adopted to investigate the effect of ADA deficiency in the brain.

Currently, we are investigating the use of a chemical inhibitor, EHNA, to inhibit ADA in a neuroblastoma cell line, with the addition of adenosine and deoxyadenosine to the cell media to model substrate accumulation in ADA deficiency. Following Annexin V / 7AAD staining, flow cytometry has been used to identify living, apoptotic and dead cell populations under different cell culture conditions. Our results show that ADA inhibition alone does not induce apoptosis; a combination of both inhibition and substrate accumulation is required.

Preliminary evidence shows volume loss and abnormalities affecting the basal ganglia and thalamic brain regions in an ADA-/- murine model. Initial experiments focus on neuroinflammation by staining brain sections obtained from ADA-/- mice treated with PEG-ADA at P15, P55 and 6 months. Mice succumb if left untreated and since PEG-ADA is unable to cross the blood brain barrier, these mice represent a KO cohort. Levels of CD68 and GFAP, markers of microglia and astrocytes respectively, were quantified and our results, although preliminary, show that microglia and astrocytes may play a role in the observed neurological abnormalities.

This is a novel project, and we intend to further elucidate the apoptotic mechanism induced by ADA inhibition and substrate accumulation in vitro. In parallel, our in vivo investigation will be extended by sequencing RNA from P15 and 6 month brains to identify novel candidates affected by ADA deficiency. Furthermore, adenosine functions as a neuromodulator and we hypothesise that accumulation may alter receptor distribution and/or expression. We will investigate this hypothesis both in vivo and in vitro.