Dr S B Keddie- ABN Clinical Research Training Fellowship

Case for Support

Investigating the pathological mechanisms of neuropathy in POEMS syndrome

Background and Importance

POEMS syndrome (polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes) is a rare multisystem disease of unknown aetiology, which causes significant disability and death.(Dispenzieri 2014) A peripheral, ascending, symmetrical sensorimotor neuropathy is the dominating characteristic of the condition.

POEMS is a rare condition with an estimated incidence of less than 1 per million. It is currently thought to affect hundreds of patients in the UK, but with the formulation of well-defined clinical diagnostic criteria, and with increasing use of laboratory biomarkers, more patients are being identified. The immune mediated, demyelinating neuropathy in POEMS disease shares similar neurophysiological and histopathological characteristics of other more common inflammatory neuropathies such as Guillain-Barré Syndrome and Chronic Inflammatory Demyelinating Polyneuropathy (CIDP).(Hadden & Hughes 2003) Inflammatory neuropathies as a whole cause significant functional impairment and pain, resulting in severe disability, loss of former occupation, and sizeable economic cost.(Panaite et al. 2013; Willison et al. 2016) POEMS syndrome is potentially the most devastating of these diseases, occurring rapidly, predominantly and profoundly affecting distal hand and foot function, and being poorly recognised and diagnosed.

The pathogeneses of other inflammatory neuropathies are understood with various degrees of certainty, whereas the mechanism of nerve damage in POEMS is very poorly characterised. Despite the disease being defined by the presence of a clonal proliferation of plasma cells, and neuropathy improving through treatment of this, there is no deposition of abnormal plasma cell components in peripheral nerve biopsies.(Hitoshi et al. 1994; Dispenzieri & Buadi 2013) This suggests other pathological markers, which appear intrinsic to the presence of the abnormal plasma cell proliferation are implicated. Levels of vascular endothelial derived growth factor (VEGF), produced in tumour cells, bone, macrophages and platelets are markedly elevated in POEMS, and correlate with disease activity.(Souza et al. 2015) However, VEGF cannot be solely responsible for disease, as trials of anti-VEGF therapy have had poor, and sometimes fatal results.(Kanai et al. 2007) Other pro-inflammatory cytokines, which act in synergy on the immune, nervous and endocrine systems appear to play a role in the pathology of POEMS. Gherardi et al found raised Interleukin-1β (IL-1 β), Interlukin-6 (IL-6) and Tumour Necrosis Factor- α (TNF- α) in the serum of POEMS patients compared to that in multiple myeloma.(Gherardi et al. 1996) The balance of cytokine-tumour-cell interactions have been studied

for many years in oncology,(Dranoff 2004) and may provide important insight into the pathology of this multi system, cancer driven process.

Until now, only studies of limited numbers of circulating cytokines have been performed in POEMS research. These have been of limited value in understanding the pathophysiological processes responsible for end organ damage, particularly neuropathy.(Kanai et al. 2012) The use of Human Induced Pluripotent Stem Cell (HiPSC) derived neurons in myelinating co-cultures offers the opportunity to study the local and direct effects of POEMS-associated cytokines on pathologically relevant tissue. See Figure 1.

I hope to build the largest POEMS database in Europe from the University College London Hospital (UCLH) POEMS clinic. The database is intended for use by multi-specialty researchers for clinical trials and therapeutics. Understanding the mechanism of neuronal destruction and death in POEMS would enable further research into focused treatments to prevent POEMS polyneuropathy, reducing the associated loss of mobility. Developing knowledge and understanding of using HiPSC derived neurones in myelinating co cultures will be invaluable to further study into inflammatory neuropathies with similar characteristics, such as GBS and CIDP.

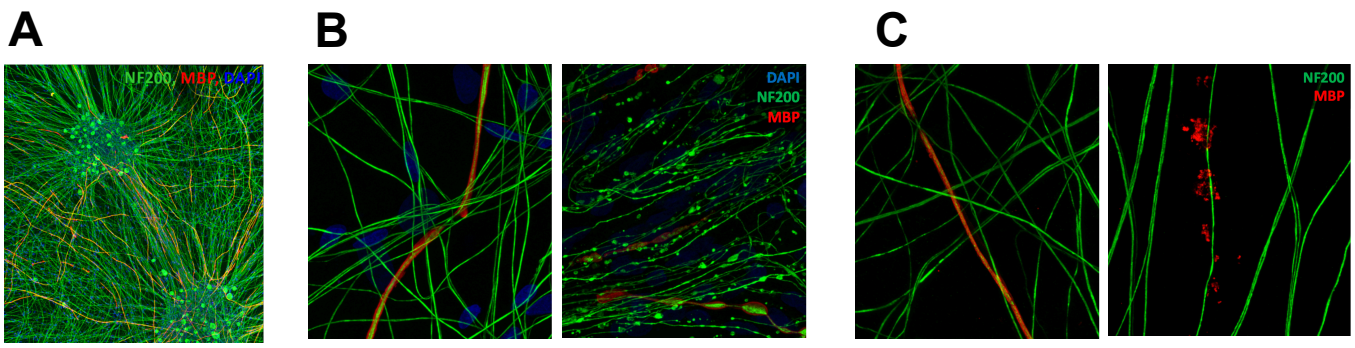


Figure 1- Myelinated and unmyelinated co-cultures, Axonal degeneration, Demyelination of Human Induced Pluripotent Stem Cell derived neurones in myelinating co-culture:

(A) Overview of myelinated co-cultures stained with NF200, MBP, DAPI. (B) Acute axonal degeneration following exposure to anti-ganglioside antibody and complement. (C) Loss of myelin without axonal loss following chronic antibody exposure in the absence of complement.

Aims

1) To characterise the clinical features of the UK cohort of patients with POEMS syndrome.

2) To define the fingerprint of cytokines and other molecules responsible for the pathogenesis of POEMS syndrome.

3) To establish the pathologic mechanisms of these molecules in causing the neuropathy associated with POEMS disease using a HiPSC derived neurones in myelinating co-culture system.

Objectives

1) Complete data entry to the 'UK POEMS Database'- including the clinical, electrophysiological, haematological and histopathological data from a series of 70 patients attending the joint neurology-haematology POEMS clinic at UCLH. This will be used to identify the POEMS phenotype, choice of treatment and response.

2) Through the use of the V-PLEX Human Cytokine 30 Plex kit, establish which cytokines and pro-inflammatory markers are present in the serum of patients with POEMS disease.

3) Identification of a POEMS neuropathy inflammasome fingerprint, to understand how inflammatory molecules are signalled, activated and regulated through use of mass-spectrometry and SIMOA

4) To produce an in vitro assay of peripheral nerve injury, through use of HiPSC derived neuronal co-cultures.

5) Identify how selected inflammatory molecules affect axonal integrity, myelination and neuronal viability of the HiPSC derived neurones in myelinating co-culture through immunostaining and live/dead assays.

Secondary objectives

1) To establish via V-PLEX Human Cytokine 30 Plex kit, whether any of the neuropathy causing pro-inflammatory markers accurately reflect disease activity or severity of accompanying plasma cell dyscrasia.

2) To consider whether a more sensitive compound biomarker can be developed as a predictor for disease activity and prognosis other than VEGF.

Research Plan

Stage 1: Creating the UK POEMS database

I am currently building this database, as part of the Rory Morrison Waldenström's Macroglobulinemia (WM) online registry, which stores all UK clinical data for WM (and other plasma cell dyscrasias). I intend to use the clinical notes to input data from all 70 of the POEMS patients under care of the Joint POEMS Clinic at UCLH, including criteria for diagnosis, neuropathy scores, laboratory and histopathology results, imaging, treatment types and responses. This will be used to deeply phenotype

the condition, and document treatment and response. It will also form the population of patients for the research study.

Stage 2: Identification of pathological markers in POEMS patients

Patients referred to the Joint POEMS Clinic attend in the morning for pre-clinic bloods and nerve conduction studies. On receiving informed consent, additional blood samples will be taken from patients for research purposes.

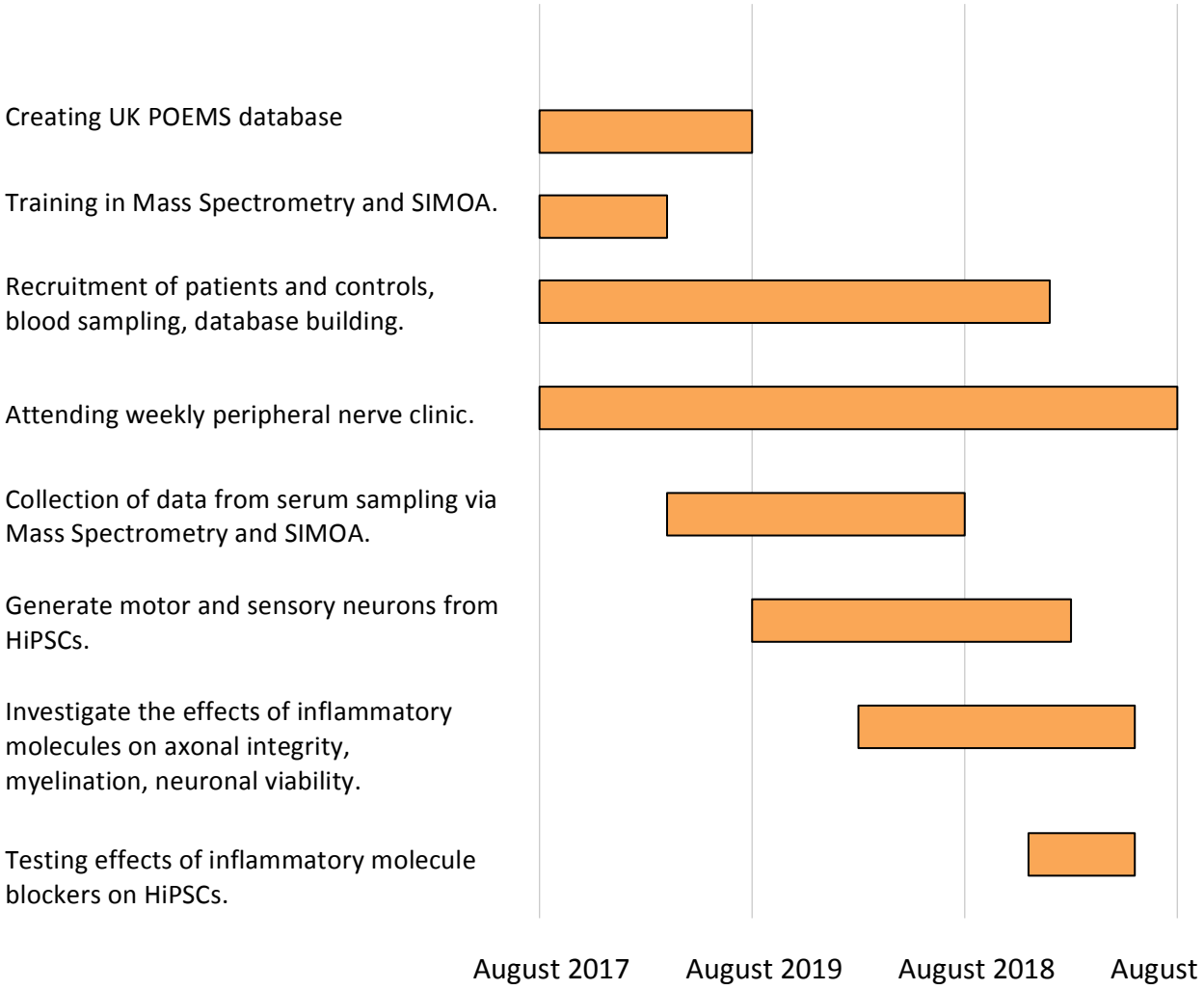
Sera from 40 pre-treatment, 40 post-treatment and 10 relapsed POEMS patients will be tested for a range of 30 common cytokines using the V-PLEX Human Cytokine 30-PLEX immunoassay kit, processed by the 'MSD Sector S600' to identify which cytokines are raised when compared to normal controls, CIDP, GBS and paraproteinaemic disorders. Mass spectrometry will be utilised for in-depth profiling of sera to capture differences in protein expression between POEMS (5 samples) and that of other inflammatory neuropathies (5 CIDP, 5 paraprotein neuropathies), plasma cell disorders (5 Waldenström's, 5 myeloma) and 'normal controls'.

Once a cytokine and proteomic fingerprint has been identified, individual cytokines and proteomic markers will be analysed across the course of a patient's disease from pre-treatment to post treatment (including some relapsed patients) to see whether cytokine levels (or other markers identified on Mass Spectrometry) relate to disease activity. SiMOA will be utilised at this stage to investigate whether early, very low concentration pathological markers can be detected in the sera of patients who relapse, prior to a rise in existing diagnostic markers, such as VEGF or reaccumulation of a plasma cell clone.

Stage 3: Establishing the pathogenic effects of inflammatory molecules implicated in POEMS disease on the peripheral nerve

The neurotoxicity of hyperacute patient sera and subsequently each relevant identified pathological cytokine (or other inflammatory markers identified) will be tested against HiPSC derived neurons in myelinating co-cultures, to identify which are responsible for peripheral nerve injury. Utilising HiPSC derived neurones will produce more accurate representations of human peripheral nerves in vivo compared to animal models, whilst allowing for large-scale replication for experimental design. Cultures will be developed under the supervision of the vastly experienced neuroimmunology group at Oxford, as part of the StemBANCC consortium.(StemBANCC 2012) They have reliably and reproducibly generated sensory neurones with efficiency in excess of 90%. Cultures take around 3 months to develop and remain stable for 9 months. The effects on axonal integrity, neuronal viability (using live/dead assays) and myelination will be assessed for each candidate molecule. Serum with specific inflammatory molecule blockers will be added to HiPSC cultures as to confirm pathogenicity.

Timeline and milestones



Exploitation and dissemination

Identifying pathological substrates in POEMS may assist in the development of a serum screening panel, which can be used in the diagnosis of POEMS patients, or in predicting relapses. Secondly, there is scope for specific inhibitory agents to be developed once the causative mechanism is discovered, such as the development of the monoclonal antibody bevacizumab for VEGF. HiPSC derived neurones in myelinating cell cultures could be developed into a commercially exploitable enterprise, giving researchers the ability to test how neurons are damaged in disease, and analyse the effects of inhibitory agents.

Research techniques and data will be presented at national and international conferences, notably the Peripheral Nerve Society, the Inflammatory Neuropathy Consortium, the Association of British

Neurologists Conference, and the UK Translational Research Conference. I also aim to present data to patients and the public through the GBS/CIDP foundation. Updates will be uploaded to the Institute of Neurology website, including the youtube video blog.

References for the research project

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