1. Progress to date

Case material

Sections from formalin-fixed paraffin-embedded tissue blocks from 17 MS cases and 11 non-MS control cases have been received from the MS UK tissue bank at Imperial College London. For each case, 10 serial sections were sent from 12 blocks containing grey matter pathology and 14 blocks in which no grey matter lesions have been detected (classified as normal-appearing grey matter; NAGM).

Immunohistochemical staining

Initially, it was planned to use the Ventana Discovery automated staining system for all immunohistochemical staining. However, initial optimisation work carried out in the Queen's University of Belfast lab demonstrated that manual immunostaining resulted in equally high levels of reproducibility and good quality staining as that produced by the automated system. Slides from all blocks have subsequently been stained manually with the following: a histochemical stain (LFB/H&E) which demonstrates general section architecture and specifically stains myelin; antibodies to allow histological classification of pathology and detection of MS grey matter lesions (MOG and HLA-DR); antibodies to demonstrate neuronal damage and loss (RT97 and SMI32): antibodies to demonstrate hypoxia and ER-stress response (D110, CHOP/GADD 153 and GRP 78/BiP).

Histological characterisation

All tissue blocks (both MS and non-MS control) have now been subject to an in-depth histological analysis and any pathological features described in detail. All grey matter lesions have been classified into Types 1-1V[1].This completes Task 1 (as detailed in grant proposal)

Analysis of ER-stress molecules

An initial semi-quantitative grading of staining for hypoxia and ER-stress-associated molecules, within both grey and white matter, has been carried out on all blocks. Representative images of staining from within, and adjacent to, different types of MS

grey matter lesions are currently being collected. Corresponding areas from NAGM and non-MS grey matter, from within the same cortical layers, are also being imaged. This will allow an in-depth quantitation of expression levels of ER-stress-associated molecules within areas of grey matter demyelination, as well as areas adjacent to pathology.

[1] *Cortical lesions have been neuropathologically classified into four type depending on their location within the grey matter. Type I are leukocortical, extending across the grey and white matter; type II are entirely intracortical; type III (the most common) are subpial and extend from the surface of the brain into the cortex, without entering the deeper layers; type IV traverse the full width of the cortex, without entering the white matter.*

2. Expenditure

The table below itemises costs to date. In our original grant submission, use of the Ventana Discovery system for tissue staining was envisaged, representing the major cost of the proposed work. However, following preliminary testing, it was found that for the cohort of tissue obtained from the UK MS tissue bank, the Ventana Discovery technology did not provide a qualitative advantage over experienced manual handling. This has given us an unexpected opportunity to consider expanding the number of sections to be examined manually during phases two and three of the project. Also, purchase of a ceramic infra-red hotplate and pressure cooker (cost in the region of €1,000) is now possible, which will significantly improve our capability for optimisation of antigen retrieval during future tissue processing in the MS research group at NUIG. Other small items of equipment being considered for purchase are a low-magnification microscope objective (1.5X, 2X, or 2.5X) for the Olympus light and fluorescent microscope and a software package which would enable capture of colour images from the same microscope (images are currently captured in grey scale from light microscope).

Interim summary of expenditure (November 09)		
Item	Cost	Comment
Within Tissue Core Technology Unit in the CCRB of Queen's University, Belfast, post mortem tissue characterisation using histological stains and antibodies	€1,400	Carried out and directed by Dr. Stephen McQuaid and Dr. Jill McMahon
Primary antibodies	€2,000	Purchase of more antibodies during phasees 2 and 3 may be necessary
Preliminary testing on Ventana system 25 tests at €28 per test (increased cost per test since time of grant submission)	€700	System found not to be superior to manual system. Manual system adopted for future staining.
Total	€4,100	

3. Abstracts submitted.

No abstracts have been submitted as this body of work has not yet been completed.

4. Workplan to completion date

Once the areas of brain containing high levels of ER-stress associated molecules have been correlated to grey matter pathology, we will examine these areas to determine whether any neurodegeneration has occurred. This will be carried out by examining the density of RT97 positive neurites (RT 97 labels all neurofilaments regardless of phosphorylation status). Such an analysis will be carried out on high power (400x) images from: (1) 10 different areas showing grey matter pathology in MS tissue; (2) 10 different areas showing upregulation of ER-stress-associated molecules in MS

tissue; (3) 10 corresponding areas (i.e. from the same cortical layers as those sampled in (1) and (2)) in NAGM; (4) 10 corresponding areas in control non-MS brain.

In areas/blocks where particular correlations are observed between ER-stress molecules and demyelination or neurodegeneration, further immunohistochemistry will be carried out to allow identification of the cell-types involved. These cell-specific markers include GFAP for astrocytes, Nogo A (or equivalent) for oligodendrocytes, CD68 for macrophages and Neurofilament for neurons. Cell-specific staining will be carried out as part of a fluorescence dual-labelling protocol which will also detect either myelin (MOG antibody) or one of the ER-stress-acssociated molecules. This work will be carried out in Galway and Queen's University of Belfast, necessitating another visit to these labs by Dr Jill McMahon. This will complete Task 2 (as detailed in grant proposal).

Statistical analysis will be carried out on all data and findings summarised and written up for publication. It is expected that colour images will greatly enhance the chances of publication of this body of work since much of it depends on detailed description of histopathological features and demonstration of correlation between ER-stress-associated molecule expression and pathology. Hence, it is planned to use some of the money awarded to allow inclusion of colour figures when submitting the findings for publication.