Characterisation of novel autoantigen fragmentation following Cell Death in Patients with Scleroderma and Raynaud's Phenomenon - Final Report

Background

Studies have demonstrated that autoantibodies can be found in the serum of over 95% of patients with systemic sclerosis (SSc). These autoantibodies are produced at an early stage of disease and correlate with specific clinical manifestations, helping to subgroup homogeneous patients within the broad scleroderma spectrum. Whilst it remains unclear whether the SSc specific autoantibodies are pathogenic or a simple epi-phenomenon, studies have demonstrated clear links between autoantibodies and disease. Anti-endothelial cell (EC) autoantibodies have been reported in SSc and are thought to induce apoptosis. This non-inflammatory form of cell death is a highly important mechanism of cellular housekeeping, functioning to eliminate senescent, malignant or infected cells.

Autoantibodies have also been demonstrated to most frequently target complexes of polypeptides with nucleic acids. This association with DNA and RNA has led to the hypothesis that nucleic acids may have a role in breaking immune tolerance, possibly as a result of their modification following cell death. Similarly modification of polypeptides following cell death has been strongly implicated in the generation of autoantibodies Autoantibody targets have increased immunoreactivity when cleaved into smaller fragments following the induction of apoptosis in various cell types. This may be due to exposure of cryptic epitopes or the generation of novel epitopes through protein modification. The autoantigen fragmentation pattern of targeted polypeptides has been shown to vary with type of cell death and cell type, further studies in this area may potentially help to provide additional insight into disease pathogenesis.

Aims of the Project

1. To determine the fragmentation pattern of target autoantigens identified by Scleroderma and Raynaud's sera in response to apoptosis and necrosis, and to assess whether these patterns correlate with specific clinical manifestations or disease severity
2. To determine the identity and frequency of nucleic acid (RNA) and RNA binding proteins precipitated by SSc sera in apoptotic and necrotic cells and determine their correlation with disease manifestations
3. To continue to provide a centre of excellence for autoantibody assessment in collaboration with other major UK SSc research groups

Results

Sera from patients with scleroderma, healthy controls and other connective tissue diseases were screened for autoantibody specificity using the gold-standard immunoprecipitation (IPP) method using radiolabelled K562 cell extract. Whilst this method is extremely sensitive, the use of radioisotopes and the length of time required for each assay, demonstrates a clear need for an alternative assay. Dr North has successfully been able to develop an assay using biotinylated K562 cell extract in place of radiolabelled extract and has used this to screen the same set of serum samples. With the exception of a couple of weak myositis specific autoantigens, all antigens identified using radiolabelled IPP method can be detected with the new technique, resulting in a viable alternative to the use of radioactivity.

Dr North has also developed a model for apoptotic cell death using annexin v antibodies and fluorescence activated cell sorting of K562 and human dermal endothelial cells. Different agents have been screened to induce cell death in K562 cells, with hydrogen peroxide being chosen as the most potent agent for use as a positive control.

Following the autoantibody screening, sera from 20 SSc patients (10 patients with anti-centromere autoantibodies and 10 patients with anti-Topo isomerise 1 autoantibodies), 12 lupus patients and healthy controls, were separated into IgG and IgM subclasses by chromatography. Ig fractions were subsequently qualitatively assessed by gel electrophoresis. These sub-fractions were used in assays to investigate whether pathogenicity is associated with a particular antibody subclass and later to identify target molecules. Using the apoptotic cell death model and the autoantibody subclasses, Dr North has demonstrated that IgG and IgM from individuals without autoimmune disease do not induce apoptosis in dermal endothelial cells. However, 2/6 IgG samples from ACA positive sera from patients with SSc and 1/3 IgG samples from sera derived from SLE patients induced apoptosis of dermal endothelial cells. Similarly with the IgM subfractions, 1/6 ACA positive sera and 1/3 SLE sera were also able to induce apoptosis of endothelial cells. These data provisionally demonstrate that IgG and IgM antibodies from some patients with SSc and SLE, but not from controls, are capable of inducing endothelial cell death.

As a separate project, Dr Zoe Betteridge and Dr Felix Woodhead (Addenbrokes / Royal Brompton) have been able to screen additional SSc cohorts for autoantibodies. These studies have lead to the identification a novel autoantibody targeting approximately 30 kDa and 53 kDa proteins in patients from both the Bath and Royal Brompton cohorts. Clinical studies on the patients with this autoantibody have demonstrated an association with lung disease and an overlap with myositis. Further studies using a combination on non-radiolabelled IPP and mass spectrometry have identified the target autoantigen

complex as EIF2B (Eukaryotic Initiation Factor 2B). These findings have since been confirmed by IPP-western blots using a commercial anti-EIF2 antibody and are currently being written-up for publication.

Additional collaborations between the Bath Institute for Rheumatic Diseases (Dr Zoe Betteridge and Dr John Pauling) and Papworth Hospital (Dr Felix Woodhead and Dr Jay Suntharalingam) have lead to initial studies on autoantibody specificities in idiopathic pulmonary arterial hypertension (IPAH) in comparison to SSc patients with associated PAH. The provisional findings have demonstrated the presence of non-SSc autoantibodies in approximately 10% of IPAH samples, potentially aiding in the diagnosis of IPAH from SSc associated PAH. Furthermore SSc specific autoantibodies were also detected in 5% of IPAH samples, demonstrating that some patients classed with IPAH may actually be SSc patients (with associated PAH) with sub-clinical SSc disease. The screening and full analysis of further IPAH and SSc associated PAH samples is currently ongoing.

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