

## Sophie Cameron Trust PhD Studentship

Functional effects of anti-basal ganglia antibodies in patients with encephalitis lethargica and related disorders associated with streptococcal infection.

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### 1. Significance of the proposed research

Encephalitis lethargica (EL) affected a large number of people in the pandemic in the early 1900s (von Economo, 1930). Whilst it is now considered a rare disorder it still occurs sporadically. Its frequency, presentation, disease course, treatment response and causes are unknown. Typically, EL presents as an acute illness in young adults with initial neuropsychiatric features, sleep disturbance and movement disorders. Recent cases have also included children, sometimes with a more benign prognosis than in adults. Histological and biochemical data suggest that autoimmune mechanisms play an important role in this disorder and recently we have detected serum anti-basal ganglia antibodies in affected sporadic cases associated with evidence of recent streptococcal infection (Dale et al., 2004a). ABGA are also associated with other neuropsychiatric disorders including Sydenham's chorea, PANDAS, Tourette syndrome (TS) and obsessive compulsive disorder (OCD). As ABGA are strongly associated with recent streptococcal infection this group of disorders is a good model for the study of molecular mimicry and autoimmunity. Preliminary data suggest that ABGA have effects on neuronal function and may therefore be directly involved in the pathogenesis of basal ganglia dysfunction. As auto-antibody mediated diseases respond to immunomodulatory therapy, identifying and defining the pathogenesis of these disorders is important so that patients can be appropriately treated. Preliminary evidence in children suggests that antibiotic prophylaxis may affect the natural history of these disorders and, if this proves to be the case, this will have major public health implications. Establishing this group of disorders as a "true" autoimmune disease of the CNS and demonstrating the ABGA have functional effects will establish a new paradigm.

### 2. Details of the proposal

#### 2.1. Aim and Objectives

**Aims:** To use recombinant human gamma and alpha-enolase (45-46 kDa), aldolase C (40 kDa) and pyruvate kinase (60 kDa) to investigate the functional effects of ABGA from patients diagnosed with EL.

**Primary objectives:** To use the recombinant proteins to (1) to affinity purify antigen-specific auto-antibodies to investigate the functional effects of ABGA in primary neuronal cell cultures; (2) to determine if disease can be induced by passive transfer of these antibodies to animals; (3) to attempt to induce disease in animals by inoculating animal with the individual antigens and cocktails of the relevant antigens.

#### 2.2. Hypotheses

- Autoantibodies affinity-purified from patients with EL and ABGA using the recombinant proteins will have functional effects in vitro, e.g. in the case of our identified putative autoantigens ABGA will affect neuronal function and survival.
- The passive transfer of ABGA to animals will result in stereotypical movements similar to those seen in human subjects with ABGA-associated neuropsychiatric disorders.
- Inoculating mice with these antigens in complete Freund's adjuvant will induce the development of ABGA in mice and result in stereotypical movements similar to those seen in human subjects with ABGA-associated neuropsychiatric disorders.

#### 2.3. Background

We have recently identified patients presenting with an encephalitic illness with a subsequent clinical course reminiscent of encephalitis lethargica with ABGA and evidence of recent streptococcal infection (Dale et al., 2001; Dale et al., 2004a). ABGA recognise four main protein bands of 40, 45, 60 and 98 kDa. We have purified and successfully identified all the major antigens using 2-D gel electrophoresis and mass spectrometry (Dale et al., 2006). The 45 and 98 kDa antigens are monomeric and dimeric forms of enolase, the 40 kDa antigen is aldolase C (neurone specific) and the 60 kDa antigen is pyruvate kinase M1. All the antigens are glycolytic enzymes involved in energy homeostasis, and as expected are found in the cytosol. These proteins are also located on the neuronal surface (Lim et al., 1983; Nakajima et al., 1994), where they appear to have "moonlighting" or alternative functions; e.g. enolase located on the surface of neurones acts as a receptor for plasmin/plasminogen (Pancholi, 2001) and has been shown to be a

trophic factor for neurons (Hattori et al., 1995). Plasminogen binding to neuronal surface enolase also provides trophic support to mesencephalic dopaminergic neurons (Nakajima et al., 1994). Membrane neuronal aldolase provides local membrane energy and is enzymatically active (Bulliard et al., 1997). It also forms an oxidoreductase complex with enolase and other proteins on the neuronal membrane and is thought to monitor oxidative stress and induce an appropriate cellular response (Bulliard et al., 1997). Aldolase binds tightly with ATPase protein pumps on the plasma membrane allowing direct coupling of glycolysis to the proton pump (Lu et al., 2001). The monomer of pyruvate kinase acts as thyroid hormone (T3) binding protein. Binding of T3 to pyruvate kinase inhibits enzymatic activity, suggesting that this process may be centrally involved in the control of some cellular metabolic effects induced by thyroid hormones (Kato et al., 1989). Interestingly, hyperthyroidism is a well-described cause of chorea. Membrane glycolysis provides a preferential source of ATP in order to maintain myocyte K<sup>+</sup> channels (Weiss & Lamp, 1987), ATPase and calcium uptake (Hardin et al., 1992) and Na<sup>+</sup> K<sup>+</sup> pumps on intestinal cells (Dubinsky et al., 1998). The maintenance of these pumps may be directly linked to functionally compartmentalised ATP to ADP ratios on the cell membrane (Dubinsky et al., 1998). In summary, therefore, membrane glycolytic enzymes are closely involved in the energy provision and maintenance of ion channels on the neuronal membrane, trophic support and other functions. Therefore disrupting their activity is likely to lead neuronal dysfunction.

All three of the major candidate autoantigens have protein homologues in streptococci. Interestingly, streptococcal enolase is also found on the surface of the bacterium and appears to function as an efficient plasmin(ogen) binding protein which influences tissue invasiveness and pathogenicity (Pancholi and Fischetti, 1998). The streptococcal surface enolase antibodies appear to recognise a shared epitope with neuronal surface and cytoplasmic enolase. We propose therefore that these antibodies may have a direct effect on neuronal function.

An important question is whether or not ABGA are directly involved in the pathogenesis of these disorders or simply diagnostic markers. Two studies investigating the effects of infusing serum immunoglobulin from patients with PANDAS into rat striatum found an increase in stereotypical movements compared to control antibodies (Hallett et al., 2000; Taylor et al., 2002). However, another group using the same methods failed to reproduce the results (Loiselle et al., 2004) and more recently a collaborative study was also negative (Singer et al. 2005), although this study has been criticised for being methodologically flawed (Giovannoni, 2005). A controlled trial of treatment with either plasma exchange or intravenous immunoglobulin (IVIg) in children with PANDAS, demonstrated a significant improvement in motor and psychiatric symptoms for both therapies compared to placebo (Perlmutter et al., 1999). These observations and insights from the proposed treatment effects of IVIG suggest that the autoantibodies are pathogenic.

In a pilot study we have shown that polyclonal IgG purified from patients with ABGA affects neuronal cell function. Single cell fluorescence imaging studies of primary cultured cerebellar neurones exposed for up to 6 hours to IgG from a number of patients with ABGA showed depressed calcium responses following a high potassium depolarising stimulus compared with neurones exposed to non-patient control IgG. Furthermore, in some cases neurones exposed to ABGA became apoptotic, showing pyknotic nuclei and withdrawn neurites. We plan to expand these studies as part of this grant.

In summary, EL and other ABGA-associated disorders are still an emerging entity, with major implications for neuropsychiatry. There is an expanding evidence base associating ABGA with recent streptococcal infection and linking ABGA directly to the pathogenesis of these disorders. Our group is at the forefront of this research.

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