Scientific report

To establish whether alpha/gamma enolase, a putative autoantigen, plays a role in the pathogenesis of antibasal ganglia antibody (ABGA) associated disorders

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INTRODUCTION

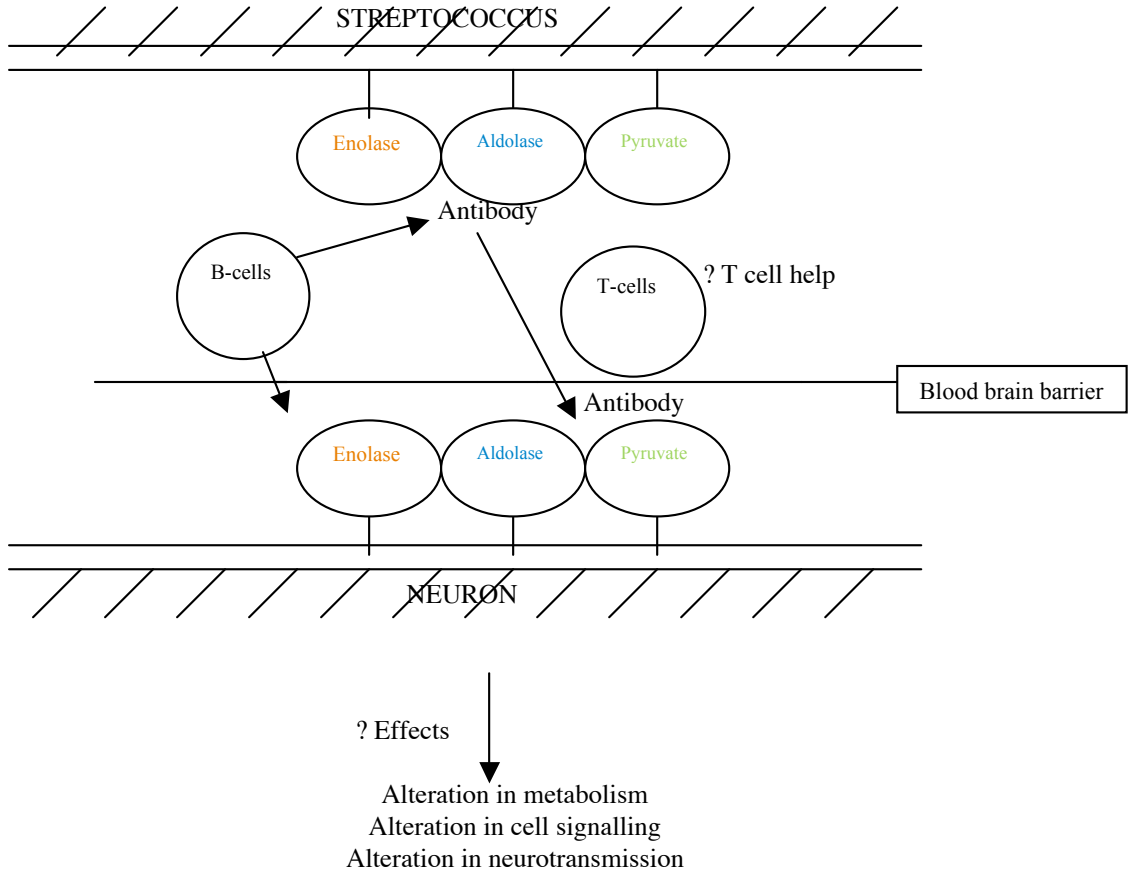
Group A streptococcus infections can result in immune-mediated brain diseases which are characterised by a spectrum of movement and psychiatric disorders. Anti-neuronal antibodies previously identified in patients, bind to a restricted group of brain antigens with molecular weights of 40 kDa, 45 kDa, 60 kDa and 98 kDa. These antigens have been defined by proteomic methods and are neuronal glycolytic enzymes. The 40 kDa, 45 kDa and 60 kDa antigens are expressed on cell surfaces and intracellularly. All these putative autoantigens have homologous proteins in Group A Streptococcus, which also expresses these glycolytic enzymes on its cell surface and share a 25-49% identity with human neuronal glycolytic enzymes. This suggests that perhaps autoimmune cross reactivity may be the pathogenic mechanism in post streptococcal CNS disease. Two 45 kDa isoforms exist; the 98 kDa band has been sequenced and is a heterodimer of these 45 kDa band isoforms. The 98 kDa band is of particular interest in that there is preliminary evidence that it is exclusively expressed in the heart and the brain. Thus the 98 kDa band may explain the link between the brain and the heart in Sydenham's chorea (i.e., why patients with streptococcal-associated acute rheumatic fever develop Sydenham's chorea). In addition, Giovannoni and colleagues have noted that encephalitis lethargica (EL; a neuropsychiatric syndrome) patients who were positive for the 98 kDa band tended to have more clinically severe forms of EL. Preliminary denaturing Western immunoblot experiments have revealed that the 98 kDa antigen is expressed in neurons, but whether this is in the cytosol or membrane is not clear. The aim of this study was to try to establish whether the 98 kDa band has a pathological role in neuropsychiatric disorders by performing a native western immunoblot (which will maintain intact structure of membranous proteins) and seeing whether purified 98 kDa positive patient IgGs have effects on energy metabolism and apoptosis in neuronal cells.

BACKGROUND

Streptococcus pyogenes (Group A beta haemolytic streptococcus, GABHS) is a pathogen in humans capable of inducing a spectrum of autoimmune sequalae. The classic prototype for a post infectious movement disorder is Sydenham's chorea (SC), where chorea and psychiatric disturbances develop after a preceding streptococcal infection (Husby et al 1976). It has been postulated that SC is the result of a cross-reactive response between anti-streptococcal antibodies and antigens of the basal ganglia (Cunningham 2000, Swedo 1993). In patients with Sydenham's chorea serum antibodies against basal ganglia structures can exist, so-called anti-basal ganglia antibodies (ABGAs). ABGAs are autoantibodies that cross react with human brain tissue (Martino and Dale 2006).

There are four main bands that ABGA's recognise; 40, 45, 60 kDa bands (Dale et al 2004) and a 98kDa band (Ueta et al 1994). All antigens are glycolytic enzymes that are also expressed on the surface of Streptococcus. They share an approximate 25-50% homology with human glycolytic enzymes, thus presenting a theoretical molecular mimicry model – see figure 1.

Figure 1 Putative autoantigens in molecular mimicry model



Defined human autoantigens have been derived from western immunoblotting and proteomic methods; 40 kDa (aldolase C), 45 kDa (neuron specific gamma enolase) 45 kDa (non-neuronal alpha enolase) 60 kDa (pyruvate kinase M1) and the 98 kDa (neuron specific alpha/gamma dimer). Enolase enzymes exist mainly as dimers (i.e gamma/gamma, alpha/alpha and alpha/gamma forms) (Marangos PJ et al 1978, Keller et al 1994, Ueta H et al) although it should be noted that monomer forms exist too on neuronal surfaces (Nakajima K et al 1994).

Candidate autoantigens are, in summary, isoforms of the neuronal glycolytic enzymes enolase, aldolase and pyruvate kinase (except alpha enolase which is non-specific in that it is widely expressed and is not enriched in the CNS).

Glycolytic enzymes not only exist in the cytoplasm, but are also present on neuronal surface membranes (Lim L et al 1983, Leung TK et al 1987, Nakajima K et al 1994, Builliard et al 1997) and there is further evidence that these enzymes are expressed in synaptic plasma membranes (Ueta et al 1994, Bahler et al 1991, Gali et al 1981). Hence autoantibodies are likely to have a greater pathogenic potential if their targets are on a cell surface rather than intracellular (Lang B et al 2003).

Western blotting provides a qualitative method for detecting ABGAs. It has been demonstrated using western blots that all patients with acute Sydenham's chorea have detectable ABGAs compared to only a 12% and 4 % detection rate in cases with rheumatic fever without SC and healthy paediatric controls, respectively (Martino and Dale 2006). Therefore, ABGAs could potentially be a diagnostic marker alongside the clinical presentation and of course other investigations (i.e. streptococcal infection detection and MRI).

Church et al (2004) demonstrated that a group of 40 children presenting with post GABHS infection neuropsychiatric disorders could be discriminated from controls (healthy children, children with simple streptococcal infections and children with other neurological/autoimmune disorders) by their ABGA status. This demonstrates that ABGAs may be clinically relevant and raises the possibility that ABGAs could generate

additional variants/subgroups deviating from the usual pathological phenotype. For example, in post streptococcal ADEM there is a higher incidence of dystonias and behavioural problems (Dale et al 2001), hence the post streptococcal form of ADEM may be phenotypically different from other forms of ADEM.

Anti-basal ganglia antibodies seem relatively specific in identifying the emerging group of post streptococcal neuropsychiatric disorders. The pathological importance of their targets is still to be fully determined. Nevertheless many disorders have been temporally related to GABHS, and ABGAs have been detected which target the same autoantigen in these disorders. There is an overlap in the clinical phenotype between the post streptococcal neuropsychiatric disorders. Perhaps these disorders represent one disease entity, but for now the experimental evidence to categorise these disorders as autoimmune is incomplete.

ABGAs have so far been detected in many neuropsychiatric disorders including; Sydenham's chorea, obsessive-compulsive disorder, Tourette's syndrome and adult-onset movement disorders amongst others.

RESULTS

Results show that 98 kDa bands are present in the soluble S1 and insoluble P1 and P2 fractions. Therefore it is likely that the 98 kDa band is both cytoplasmic and membrane-bound and thus may have functional effects. It should be mentioned that the insoluble fractions contain nuclei and cytoskeletal fractions as well as membranous structures. Nevertheless the association of the 98 kDa band with membranes, nuclei and cytoskeletal fractions as well as the cytosol suggests non glycolytic functions for the alpha/gamma enolase dimer. Perhaps alpha/gamma enolase is a 'moonlighting protein'. The concept that one gene codes for one protein which then exhibits one function is now viewed to be too simplistic given that increasing numbers of proteins are found to have two or more different functions. As a result, such proteins increase cellular complexity. With regard to the functions of the alpha/gamma dimer my results support the idea that the 98 kDa dimer may represent a link between RF and SC (Ueta et al 1994, Fontan et al, 2000). The M proteins are the specific cell wall antigens of group A streptococci. Currently the widely held view is that antibodies are generated by the adaptive immune system against the M proteins and may then cross react with cardiac myofiber protein myosin (Fae et al, 2006). The 98 kDa alpha gamma dimer represents a better antigen for explaining the link between rheumatic fever and Sydenham's chorea than the M protein hypothesis; especially given that myosin is an intracellular protein, whereas from my results the 98 kDa alpha/gamma dimer is likely to be expressed on the cell surface. This is because autoantibodies are likely to have a greater pathogenic potential if their targets are on a cell surface rather than being intracellular (Lang B et al 2003).

Figure 2 Reactivity of commercial anti-enolase antibody and purified 98 kDa positive patient IgGs

	Commercial antibody reactivity			**Sera derived antibody reactivity**		
Antigen	S1	P1	P2	S1	P1	P2
alpha	+	-	+	+	-	-
gamma	+	-	+	+	-	-
alpha-gamma	**-**	**-**	**-**	**+**	**+**	**+**

Figure 2 summarises the reactivity of both the commercial anti-enolase antibody and the 98 kDa positive patient sera. The commercial anti-enolase antibody does not recognize epitopes of the 98 kDa alpha/gamma dimer. This suggests that perhaps there is a change epitope expression when the two isoforms of enolase (alpha and gamma enolase) form a dimer. Therefore, this serves as further evidence that the 98 kDa antigen may have alternative functions than just acting as a glycolytic enzyme.

In this study, incubation of purified 98 kDa positive patient sera induced significant reductions in ATP levels when compared to controls in both neuronal and cardiac myocytes. This suggests that anti-alpha/gamma enolase, like anti-enolase, anti-aldolase C and anti-pyruvate kinase antibodies (Dale et al 2004), has effects on energy metabolism in neuronal cells in addition to effects on cardiac myocytes. However, whilst incubation of purified 98 kDa positive patient sera induced significant increases in apoptosis in neuronal cells, significant increases were not found on incubation with heart cells. However, it is possible that more cell samples were required as controls. The reason for the small number of controls (two) was due to time constraints as well as

initial difficulty with culturing the heart cells. Therefore I would like to repeat these preliminary experiments and plan to do so in my medical school vacation.

FURTHER WORK

In order to see whether the antibodies definitely bind to the alpha/gamma dimer, immunohistochemistry experiments involving purified 98 kDa positive patient IgGs and intact neurons need to be performed. Confocal microscopy can then be used to define the cellular distribution of the alpha/gamma dimer and thereby show whether the dimer colocalises with any ion channels. The regional distribution of the alpha/gamma dimers in the CNS and the heart needs to be investigated in order to see whether they are enriched in the basal ganglia and conducting system of the heart. Lastly, using affinity purified IgG from patients with anti-basal ganglia antibody associated disorders, co-immunoprecipitation experiments will be performed to see what membrane proteins colocalise with the alpha/gamma dimers.

The findings from the current study are positive and suggest that the alpha/gamma enolase dimer does indeed play a role in the pathogenesis of anti-basal ganglia antibody associated disorders. However, these are preliminary findings and as highlighted above further experiments need to be done. The findings here together with the future planned experiments should tell us whether the 98 kDa antigen is indeed the link between rheumatic fever and Sydenham's chorea (for example, whether the same mechanism that causes first degree heart block in rheumatic fever patients is responsible for the basal ganglia dysfunction in Sydenham's chorea).

REFERENCES

Bahler M, Klein RL, Wang JK, Benfenati F, Greengard P. A novel synaptic vesicle-associated phosphoprotein: SVAPP-120. J Neurochem. 1991 Aug;57(2):423-30

Bulliard C, Zurbriggen R, Tornare J, Faty M, Dastoor Z, Dreyer JL. Purification of a dichlorophenol-indophenol oxidoreductase from rat and bovine synaptic membranes: tight complex association of a glyceraldehyde-3-phosphate dehydrogenase isoform, TOAD64, enolase-gamma and aldolase C. Biochem J. 1997 Jun 1;324 (Pt 2):555-63.

Church AJ, Dale RC, Giovannoni G. Anti-basal ganglia antibodies: Diagnostic utility in suspected post-streptococcal movement disorders? Arch Dis Child. 2004 Jul;89(7):611-4

Cunningham MW (2000) Pathogenesis of group A streptococcal infections. Clin Microbiol Rev. 13: 470-511.

Dale RC Church RJ Cardosso F et al(2001) Post streptococcal acute disseminated encephalomyelitis with basal ganglia involvement and auto reactive anti basal ganglia antibodies. Annals in Neurology. 50:588-591

Dale RC, Candler PM, Church AJ, Wait R, Pocock JM, G Giovannoni (2004a) Glycoclytic enzymes on neuronal membranes are candidate autoantigens in post streptococcal neuropsychiatric disorders. Movement disorders. Volume 19 Supplement 9 S33.

Fae KC, da Silva DD, Oshiro SE et al (2006) Mimicry in recognition of cardiac myosin peptides by heart-intralesional T cell clones from rheumatic heart disease. Journal of Immunology. 176(9): 5662 - 5670.

Fontán PA, Pancholi V, Nociari MM, Fischetti VA(2000) Antibodies to streptococcal surface enolase react with human alpha-enolase: implications in poststreptococcal sequelae. J Infect Dis. 182(6):1712-21

Gali P, Hauw JJ, Boutry JM, Hartmann L. Immunofluorescence and histochemical methods for neural M1 pyruvate kinase localization. J Neurochem. 1981 Dec;37(6):1377-84.
Garvey M, Giedd J, Swedo S (1998) PANDAS: The search for environmental triggers of paediatric neuropsychiatric disorders. Lessons from Rheumatic Fever. Journal of Child Neurology. 13: 413-423.

Husby G, Van de Rijn I, Zabriskie JB et al (1976) Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. Journal of Experimental Medicine. 144: 1094-1110.

Lang B, Dale RC, Vincent A. New autoantibody mediated disorders of the central nervous system. Curr Opin Neurol. 2003 Jun;16(3):351-7.

Leung TK, Hall C, Monfries C, Lim L (1987) Trifluoperazine activates and releases latent ATP-generating enzymes associated with the synaptic plasma membrane. J Neurochem. 49(1):232-8.

Lim L, Hall C, Leung T, Mahadevan L, Whatley S (1983) Neurone-specific enolase and creatine phosphokinase are protein components of rat brain synaptic plasma membranes. J Neurochem. 41(4):1177-82.

Marangos PJ, Zis AP, Clark RL, Goodwin FK. Neuronal, non-neuronal and hybrid forms of enolase in brain: structural, immunological and functional comparisons. Brain Res. 1978 Jul 7;150(1):117-33.

Martino D, Dale RC (2006) Post-streptococcal autoimmune brain disorders. In: David T, ed. Recent advances in paediatrics 23. London: RSM Press,17–32.

Nakajima K, Hamanoue M, Takemoto N, Hattori T, Kato K, Kohsaka S. Plasminogen binds specifically to alpha-enolase on rat neuronal plasma membrane. J Neurochem. 1994a Dec;63(6):2048-57.

Swedo SE, Leonard HL, Schapito MB, Casey BJ (1993) Sydenhams chorea: physical and psychological symptoms of St Vitus dance. Paediatrics. 91: 706-713.