

SCIENTIFIC COMMENTARY

Stiff, twitchy or wobbly—are GAD antibodies pathogenic?

Over the last decade or so there has been increasing recognition of the importance of measuring antibodies to neuronal proteins in the diagnosis and management of disease. Such antibodies can be classified into two main groups. First, there are those that are markers for a disease process, such as the paraneoplastic antibodies against Hu, Yo, Ma2, but which are not thought to cause the disease. This is because their targets are cytoplasmic or nuclear proteins that are not exposed on the surface of the neurons, and because the patients do not respond to immunotherapies such as plasma exchange, intravenous immunoglobulins or corticosteroids. Moreover, the antibodies do not necessarily associate with specific syndromes; for instance, Hu antibodies can be found in a wide range of disorders from neuropathy to limbic encephalitis. By contrast, the second group includes those that are not only diagnostic biomarkers but are also pathogenic (or likely to be). These bind to important membrane proteins, their epitopes exposed on the outside of the target cells; they are usually syndrome specific, e.g. the acetylcholine receptor antibodies in myasthenia gravis and, most importantly, the patients do well with treatments that reduce the levels of the antibodies.

Where do glutamic acid decarboxylase (GAD) antibodies, that are found in diabetes and stiff-person syndrome (SPS), fit into this scheme? GAD is a cytoplasmic enzyme and there is no evidence that it is expressed on the cell surface; even in type 1 diabetes mellitus (DM1) GAD antibodies are thought to be markers of pancreatic destruction rather than pathogenic (Ellis and Atkinson, 1996). Moreover, GAD antibodies are found not only in SPS (Solimena *et al.*, 1990) but also in cerebellar ataxia (Honnorat *et al.*, 2001) and in some cases of epilepsy (Peltola *et al.*, 2000; McKnight *et al.*, 2005). On the other hand, SPS does respond to immunotherapies such as intravenous immunoglobulin (Dalakas *et al.*, 2001) and other drugs (see Dalakas, 2008). So are GAD antibodies themselves pathogenic, or are they markers for one or more autoimmune processes that associate with different clinical syndromes?

The article in this issue by Albert Saiz and colleagues from Barcelona does not address the pathogenicity of GAD antibodies directly, but does make some interesting observations. They took sera that were positive for GAD antibody reactivity on routine paraneoplastic antibody testing and determined that their titres were all >2000 U/ml

by a radioimmunoprecipitation method. They then matched these sera with those of similar titres identified in an endocrinology laboratory, and obtained clinical details and cerebrospinal fluid data on the patients. Confirming previous reports, the most frequent clinical syndromes were SPS and cerebellar ataxia but there were also cases of epilepsy, limbic encephalitis and myasthenia, and four had tumours. Interestingly, three of the 14 high GAD antibody patients identified by the endocrinologists had long-standing epilepsy (rather than SPS or cerebellar ataxia) and the others did not have neurological disease. The authors rightly conclude that high GAD antibodies can be associated with several neurological syndromes, and that high titres are not invariably associated with neurological diseases. Importantly, the presence of high GAD antibodies does not exclude a paraneoplastic neurological condition, and tumours of neuroendocrine origin, in particular, should be considered.

The most interesting, and perhaps contentious, conclusion from this study concerns the findings in cerebrospinal fluid. Oligoclonal bands were found in 35% of the SPS and 69% of the cerebellar ataxia patients, and both groups had evidence of intrathecal synthesis of GAD antibodies. The authors claim that CSF studies should be done on anyone with high GAD antibodies and a coexistent neurological syndrome, in order to confirm that 'the syndrome is related to GAD autoimmunity'. In my view, the concept that intrathecal synthesis equates with an immune-mediated pathogenesis is one that needs to be explored further. Given that the main location of GAD is in the CNS, one might expect DM1 GAD antibody-specific B cells to be attracted there and stimulated to produce the antibodies, irrespective of whether the antibodies are associated with a neurological disease. Conversely, lack of intrathecal synthesis of an antibody does not, I believe, exclude autoimmunity. Many patients with voltage-gated potassium channel (VGKC) antibodies and immunotherapy-responsive limbic encephalitis do not have oligoclonal bands and their spinal fluid can be negative for VGKC antibodies (Vincent *et al.*, 2004; Jarius *et al.*, 2008).

There are some limitations. In selecting sera that were referred for paraneoplastic antibodies and positive by immunohistochemistry for GAD antibodies, there was not only a possible bias towards patients with paraneoplasia, which could explain the relatively high rate of cerebellar

ataxia and paraneoplastic disorders, but also a potential bias against patients whose serum did not show reactivity by immunohistochemistry, but who might also have neurological disorders. Our experience of screening neurological patients directly with the radioimmunoprecipitation assay indicates a surprisingly large number of patients with high GAD antibodies (>1000 U/ml) and many of the sera come from neurological clinics. We have not yet done an 'audit' of the clinical syndromes and treatment responses, but anecdotal evidence suggests that GAD antibodies are more common than reported in the current study, and often a marker for an immunological process in a proportion of patients with neurological diseases. Indeed, there is growing evidence that there are other serum antibodies that can bind to cell surface determinants on neurons (Raju *et al.*, 2006; T. Chang *et al.*, in preparation) and which are therefore much more likely to be the pathogenic entity. Nevertheless, there may be patients in whom the GAD antibodies are a late manifestation of an ongoing degenerative process; this has not been excluded particularly in patients with long-standing epilepsy (Peltola *et al.*, 2000; McKnight *et al.*, 2005), as evidenced by cases described here, although one of these did appear to respond to immunotherapies.

That case apart, one of the disappointments in this otherwise informative clinical study is that there is very little about treatment responses. Most of the literature, apart from one trial of intravenous immunoglobulin (Dalakas *et al.*, 2001), relates to small studies or single cases, and treatments have often been initiated many years after the onset of the disorders; this should not have been the case in many of the patients identified here. What is needed now is unbiased patient identification, a more structured approach to the study of the clinical associations, a systematic study of the relevance of spinal fluid findings in patients with or without neurological diseases, and

follow-up data after appropriate treatments. The study of GAD positivity could tell us a great deal about the different roles of antibodies in CNS diseases.

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References

- Dalakas MC. Advances in the pathogenesis and treatment of patients with stiff person syndrome. *Curr Neurol Neurosci Rep* 2008; 8: 48–55.
- Dalakas MC, Fujii M, Li M, Lutfi B, Kyhos J, McElroy B. High-dose intravenous immune globulin for stiff-person syndrome. *N Engl J Med* 2001; 345: 1870–6.
- Ellis TM, Atkinson MA. The clinical significance of an autoimmune response against glutamic acid decarboxylase. *Nat Med* 1996; 2: 148–53.
- Honnorat J, Saiz A, Giometto B, Vincent A, Brieva L, de Andres C, et al. Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. *Arch Neurol* 2001; 58: 225–30.
- Jarius S, Hoffmann L, Clover L, Vincent A, Voltz R. CSF findings in patients with voltage gated potassium channel antibody associated limbic encephalitis. *J Neurol Sci* 2008; 268: 74–7.
- McKnight K, Jiang Y, Hart Y, Cavey A, Wroe S, Blank M, et al. Serum antibodies in epilepsy and seizure-associated disorders. *Neurology* 2005; 65: 1730–6.
- Peltola J, Kulmala P, Isojarvi J, Saiz A, Latvala K, Palmio J, et al. Autoantibodies to glutamic acid decarboxylase in patients with therapy-resistant epilepsy. *Neurology* 2000; 55: 46–50.
- Raju R, Rakocevic G, Chen Z, Hoehn G, Semino-Mora C, Shi W, et al. Autoimmunity to GABAA-receptor-associated protein in stiff-person syndrome. *Brain* 2006; 129: 3270–6.
- Solimena M, Folli F, Aparisi R, Pozza G, De Camilli P. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. *N Engl J Med* 1990; 322: 1555–60.
- Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 2004; 127: 701–12.