Diagnosis

The need for caution in considering the diagnostic utility of antibasal ganglia antibodies in movement disorders

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Commentary on the paper by Church *et al* (see page 611)

> ''Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning.'' **(Sir Winston Churchill, Speech in November 1942)**

In this issue, Church and colleagues discuss the possibility that immunological analyses of sera could serve as diagnostic markers for movement disorders associated with streptococcal infection.[1] These investigators have been at the forefront of studies evaluating antibasal ganglia antibodies (ABGA) in a variety of proposed poststreptococcal movement conditions. These studies have great potential, given that identification of a specific immune mechanism could lead to insights into pathophysiological mechanisms as well as the development of new therapies. Nevertheless, based on ongoing studies in our laboratory, we feel compelled to raise concerns about the reproducibility of ABGA results, about methodology, and about the inability to confirm serum microinfusion induced alterations of rodent behaviour. As described in the quotation from the wise leader and historian Winston Churchill, we believe that whereas important initial steps have been taken, the final solution has not been reached. The goal of this commentary is not to criticise existing reports, but to identify disparities in data and to emphasise the need for additional testing, before implementing ABGA screening for children with movement disorders.

HYPOTHESIS AND REQUIREMENTS FOR CONFIRMATION

In susceptible individuals, antibodies produced against the group A β haemolytic streptococcus (GABHS) cross-react with epitopes on neurones located in the basal ganglia, through a process of molecular mimicry, and cause movement abnormalities (chorea, tics, other). IAcceptance of the aforementioned poststreptococcal autoimmune hypothesis requires confirmation on two major levels: (1) epidemiological evidence showing a clear association between streptococcal infection and movement disorders; and (2) definitive evidence for an autoimmune mechanism. Since the focus of this commentary is on laboratory based approaches, the reader is referred to other sources for a discussion of clinical issues and requisites for documentation of a streptococcal infection.[2–4] Experimental affirmation of autoimmunity requires several factors, including the identification of autoantibodies, the presence of immunoglobulins at the pathological site, the induction of symptoms with autoantigens, passive transfer of the disorder to animal models, and a positive response to immunomodulatory therapy.[5]

METHODS FOR EVALUATING ABGA

ABGA have been measured in children with SC and TS by either enzyme linked immunosorbent assays (ELISA) or immunofluorescent methods. ELISA is generally selected because it is more specific than immunofluorescent techniques.[6] Western blot analyses, which permit detection of autoantibody activity against specific brain epitopes, have also been applied successfully. Lastly, the bilateral microinfusion of sera or IgG into rodent striatum has been suggested as a valuable method to assess the functional effect of ABGA obtained from individuals with movement disorders. Although each of the ABGA detection methods appears to be relatively straightforward, results are affected by the use of sera or IgG, brain region (caudate, putamen, or globus pallidus), condition of tissue (fresh or frozen), method of tissue preparation (with or without removal of lipids, that is, delipided), selection of tissue fractions (supernatant, pellet, or synaptosomes), method for Western blotting (electrochemiluminescence, ECL, or colorimetric detection), and several other factors (Singer *et al*, unpublished data).

NOTEWORTHY LABORATORY DISCREPANCIES

In their paper, Church and colleagues provide information on antibodies detected in 40 children in the UK presenting with movement disorders associated with streptococcal infections: 20 PANDAS, 16 SC, and four ''idiopathic'' movement disorders.[1] Pooled group results suggest that this cohort can be differentiated from a variety of disease controls by ELISA and Western immunoblotting methods. In contrast, results of similar immune assays performed on children with SC and PANDAS and compared to healthy controls, discussed below, have produced significantly different results.[7 8]

ELISA

In nine children with acute SC, age range 5.8–13.2 years, assays performed on fresh brain tissues from caudate, putamen, and globus pallidus showed that optical density (OD) values were higher in SC patients than controls across all brain regions, but did not reach a level of significance. Although our results (33% above a negative cutoff) did not confirm the 95% positive rates reported by Church and colleagues,[6] the documented positive trend supports the possibility that inclusion of additional cases would identify a meaningful difference in SC. In contrast, however, two separate evaluations of ELISA in children with PANDAS have shown absolutely no difference from controls[8] (Singer *et al*, unpublished data). In the first evaluation, ELISA was measured on supernatant, pellet, and synaptosomal preparations from fresh postmortem caudate, putamen, and globus pallidus by using serum from 15 children with classic PANDAS (participants in the hallmark study by Swedo *et al*[9]). This lack of correlation has been further confirmed in a second study of 40 additional cases of PANDAS. Thus, ELISA is unchanged in a population of children who have a proposed association with GABHS. Nevertheless, since ABGA are identified in normal individuals, it is possible that small changes could be missed or the serum might be obtained after symptoms have waned.

Abbreviations: ABGA, antibasal ganglia antibodies; GABHS, group A β haemolytic streptococcus; PANDAS, paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections; SC, Sydenham's chorea; TS, Tourette syndrome

Western immunoblotting

In this technique, small amounts of brain protein are subjected to electrophoresis in acrylamide gels, the protein is transferred to nitrocellulose, exposed to serum, and then to an antihuman IgG secondary antibody that is conjugated to either horseradish peroxidase or a colorimetric agent. Resulting bands are then analysed by several techniques ranging from direct visual comparison (useful with minimal bands) to digital conversion of bands on each blot to peaks with assigned molecular weights and measurable peak heights and areas.

Church and colleagues, using frozen tissue and a colorimetric assay, identified only a few bands in controls (limited reactivity against any basal ganglia antigens), but significantly more bands in poststreptococcal patients.[1] They suggest that specific MW bands (60, 45, 40 kDa) are commonly detected in their movement disorder patients but not in controls. Their identification of similar bands in subjects with different clinical disorders raises questions of disease specificity. In addition, as shown in fig 1, the use of fresh postmortem tissue and an ECL detection system identifies many more bands than shown by the method used by Church and colleagues (frozen tissue and a colorometric detection system). This suggests that the methods selected can have an important influence on the detection of potential disease specific basal ganglia antigens. A further issue is that antigen binding, claimed to be associated with PANDAS and SC patients, is also found in controls. That is, in the evaluation of fresh postmortem tissue without lipids from 10 control subjects, binding was present at 60 kDa in 100%, at 45 kDa in 80%, and at 40 kDa in 100%; binding in frozen delipided tissue was present in 100%, 30%, and 40%, respectively (Singer *et al*, unpublished data). Lastly, questions exist about the importance of a band at 60 kDa, since epitopes at this same molecular weight are identified using only secondary antibody, without the addition of serum or IgG (fig 2).

Western immunoblotting of serum from patients with SC, analysed by use of discriminant analysis of data vectors calculated from individual blots, has shown that patterns are significantly different from controls, most notably in caudate supernatant fractions. Numerous antigens contributed to this statistical difference, but the two most prominent molecular masses that distinguished the groups were at 126 and 113 kDa.[7] In PANDAS, complex staining patterns were observed in all cases, but no differences from control were seen for either the number of bands or the total density of bands. Discriminant analyses did show small differences in mean data vectors between PANDAS and controls that were limited to the caudate supernatant preparation. These changes, however, were minimal compared to those detected between SC and controls. Whether differences in Western blotting offer specific insight into the pathophysiology of movement disorders or merely represent an epiphenomenon, remains undetermined. For example, children with the diagnosis of TS and no history of streptococcal associated exacerbations show prominent immunoblot differences.[10] To date, weaknesses in most ABGA studies include the analysis of antibody patterns at only a single point in time, rather than in longitudinal samples, and the failure to determine whether there is an association between ABGA and a streptococcal infection. Lastly, it should be recognised that measured autoantibody repertoires can be secondary immune responses to components of damaged tissue[11] rather than to the inciting pathological factor.

Indirect immunofluorescence

Studies in the Church and Singer laboratories have identified fluorescent binding in some patients with TS and PANDAS. Location of this positive binding remains unclear, ranging from axonal with sparing of the cell body to astroglial processes. Additional analyses with double labelled staining (antihuman IgG with GFAP or a neuronal indicator) are necessary to further identify the precise anatomical region. Similar positive binding has also been detected in controls.

RODENT MICROINFUSION MODELS

One of the major criteria for any neurological disorder to be recognised as autoimmune is the ability to transfer the disease passively to animals. Two studies, both frequently cited as supporting evidence for an autoimmune mechanism in paediatric movement disorders, have reported behavioural effects after the infusion of ABGA from subjects with TS into rodent striatum.[12 13] That is, the microinfusion of sera containing increased antibodies into the ventral or ventrolateral striatum of rats significantly increases stereotypic behaviours (for example, licks and forepaw shakes) and in one study caused episodic utterances.[12] In contrast to the

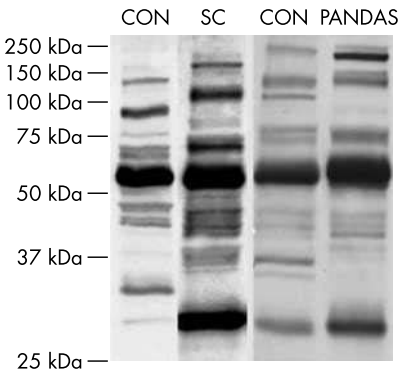

Figure 1 Western blotting using sera and caudate supernatant. Numerous bands (antigen-antibody interactions) are observed in SC and PANDAS subjects and controls.

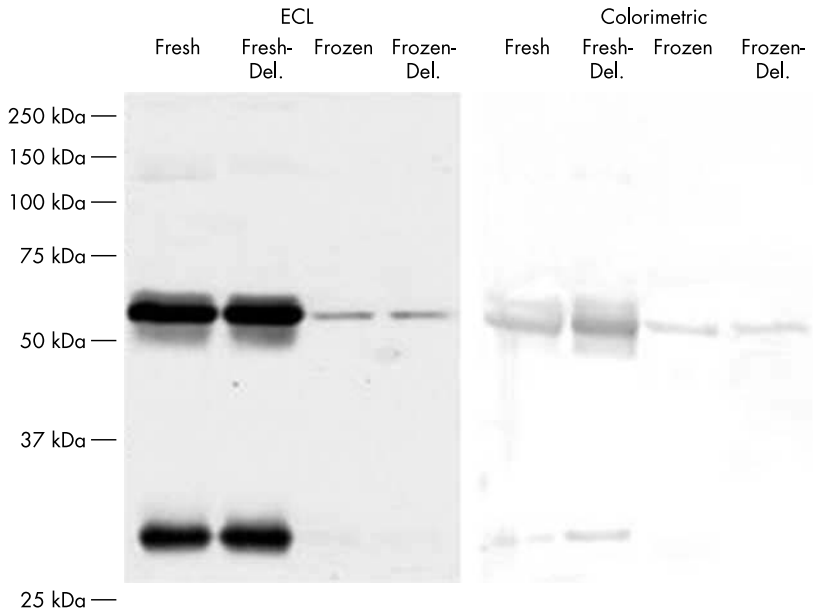

Figure 2 Two Western blotting methods using various caudate preparations and only secondary antibody. Despite the lack of serum (source of the primary antibody), bands are seen at 60 and 28 kDa. Del., delipided.

aforementioned, however, two additional studies have questioned the validity of the model. For example, microinfusion of sera from patients with TS and PANDAS into the same striatal sites as in prior reports, failed to induce any significant increase in either stereotypic behaviour or episodic utterances.[14] Lastly, a double blind, collaborative effort involving three institutions, showed no differences in behaviours between infusions of TS sera with high and low ABGA titres, but non-specific increases induced by continuous infusion (Singer *et al*, unpublished data).

CONCLUSION

In conclusion, although it is currently hypothesised that an autoimmune process is the underlying pathophysiological mechanism in several movement disorders, specific disease related brain autoantigens have not been identified. Furthermore, before accepting the pathogenic relevance of a monoclonal antibody, confirmation will require determination of regional and neuronal specificity, evidence of cross reactivity with streptococcal proteins, and documentation of passive transfer of the disorder. We believe that the proposal of an autoimmune ABGA mechanism for poststreptococcal neurological disorders deserves careful study, but emphasise that the use of ABGA as diagnostic markers is currently premature.

For clinicians considering making the diagnosis of PANDAS, we suggest strict adherence to the formal published criteria,[10] recognition that the diagnosis requires longitudinal assessments, realisation that a single measurement of antistreptococcal antibodies has limited value, and that treatment with prophylactic antibiotics or immunomodulatory therapies is controversial and fraught with potential serious side effects. Until further clarification is available, we suggest that therapy should continue to focus on the use of standard approaches to control symptoms. Along with the scientific community, we anxiously await the result of longitudinal case-control studies now in progress.

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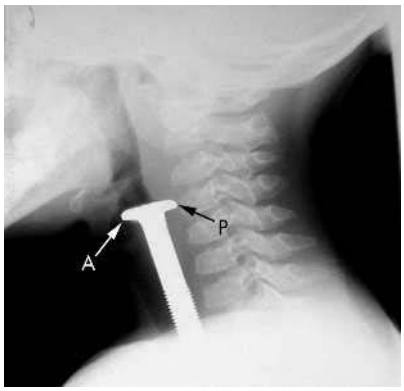
IMAGES IN PAEDIATRICS

A novel cause of neck stiffness



A 4 year old boy was referred to hospital with suspected meningitis. There was a four day history of neck stiffness and anorexia but also of drooling and dysphonia. Examination revealed him to be significantly dehydrated but alert and afebrile. There was no stridor. Serum urea and electrolytes were consistent with hypernatraemic dehydration: sodium 161 mM/l; urea 16.9 mM/l; creatinine 74 μM/l. A lateral radiograph of the neck showed a large foreign body in the proximal oesophagus at the level of cricopharyngeus (see fig). After appropriate fluid resuscitation, the foreign body was removed under general anaesthesia revealing a significant ulceration in the posterior superior pharynx and minor oedema of the arytenoids corresponding with P and A on the figure respectively. The child's parents identified the foreign body as a bolt recently noted to be absent from its hole in a bed-head. The postoperative recovery was swift and complete. The discharge weight was almost 15% greater than the weight on presentation. This case illustrates how cricopharyngeus (''the upper oesophageal sphincter'') is one of the three sites where oesophageal foreign bodies may lodge—the other two sites being where the aortic arch descends anterior to the mid oesophagus and at the level of the cardiac sphincter. The case also serves as a reminder that young children can conceal a potential life threatening condition for several days.

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