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Antineuronal antibodies in a group of children with obsessive–compulsive disorder and Tourette syndrome

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Abstract

An autoimmune hypothesis has been suggested for early onset obsessive–compulsive disorder and Tourette syndrome. The term: *Paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection* (PANDAS) has been proposed as an aetiological subtype of OCD and TS, related to a Group A beta haemolytic streptococcal (GABHS) infection that triggers an autoimmune response. Antineural antibodies have been studied and found in the sera of some patients with these disorders, and they are thought to cross-react with streptococcal and basal ganglia antigens. The present study included 32 prepubertal-onset OCD patients, 21 with TS diagnosis (some of them meeting criteria for PANDAS) and 19 normal children, all aged between 9 and 17 years. Antibodies were assayed by immunohistochemistry and immunoblot. Special attention was paid to the methodology and a high serum dilution was used to minimize non-specific binding. No anti-basal ganglia antibodies were detected by immunohistochemistry in any of the samples. Two proteins, with approximate molecular weights of 86 kDa and 55 kDa, were found in sera from 7 patients. Though the study supports the hypothesis of an autoimmune process underlying OCD or TS in some patients, further research is needed.

Keywords: Antineuronal antibodies; PANDAS; Autoimmunity; Obsessive–compulsive disorder; Tourette syndrome

1. Introduction

Early onset obsessive–compulsive disorder (OCD) is often described as a subtype of the condition with special epidemiological and clinical characteristics (Eichstedt and Arnold, 2001; Geller et al., 1998; Rapoport et al., 1992). Childhood-onset OCD is also associated with comorbid Tourette syndrome (TS). A familial relationship between TS and OCD has been demonstrated, and an autoimmune hypothesis has been suggested for both syndromes (Morer et al., 2005b). The controversial concept *paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection* (PANDAS) was proposed to describe an aetiological subtype of OCD and TS, related to a Group A beta haemolytic streptococcal (GABHS) infection that triggers an autoimmune response based on molecular mimicry (Cunningham, 2000). Interestingly, recent research indicates a possible relation between streptococcal infection and the risk of obsessive–compulsive disorder (Mell et al., 2005). However, many issues remain to be resolved before the autoimmune hypothesis can be accepted, especially questions concerning the detection of antineuronal antibodies (Church et al., 2002; Husby et al., 1976; Kotby et al., 1998; Morshed et al., 2001; Swedo et al., 1993) and specific markers (Morer et al., 2005a).

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Antineural antibodies have been found and studied in the sera of some patients with Sydenham's Chorea (SC), Tourette syndrome or tic disorder, and in obsessive–compulsive disorder, and they are thought to cross-react with streptococcal and basal ganglia antigens (Kiessling et al., 1993, 1994; Morshed et al., 2001; Singer et al., 1998). In SC, antineuronal antibodies have been detected and quantified by several methods. In a recent study, anti-basal ganglia antibodies were found in 95% of acute and 56% of persistent SC patients (Church et al., 2002). Western blot analyses detected anti-60 kDa antibodies which occurred more frequently in tic disorders or TS patients (Hoekstra et al., 2003; Singer et al., 1998; Trifiletti and Packard, 1999). Recently, positive anti-basal ganglia antibodies were found in 64% of PANDAS patients but in only 9% of controls with a documented streptococcal infection but no neuropsychiatric symptoms (Pavone et al., 2004). Immunoblotting has also identified multiple bands against the caudate supernatant fraction in PANDAS with primary tics, which differed from the control group (Church et al., 2004). Few studies have specifically looked for antineuronal antibodies in OCD. Recently a consolidated group reported the presence of antibrain antibodies in 42% of an OCD group of 50 children compared with rates between 2% and 10% in three different control groups (paediatric autoimmune, neurological and streptococcal). Again, the most frequent molecular weights were 40, 45 and 60 kDa (Dale et al., 2005).

A pathogenic role for anti-basal ganglia antibodies has been suggested by two studies describing induced movements in rats after infusion of IgG of sera from patients with PANDAS (Taylor et al., 2002), but a recent multicentre study failed to show behavioural abnormalities when sera of patients with high titre antineuronal antibodies were microinfused into rat striatum (Singer et al., 2005b). Recently, it has also been reported that antibodies from an SC patient reacted against lysoganglioside and *N*-acetyl-beta-D-glucosamine, a neuronal antigen that is also found at the GABHS surface (Kirvan et al., 2003), and Dale et al. have identified antibodies against neuronal glycolytic enzymes autoantigens in 20 unselected post-streptococcal CNS patients compared to 20 controls (Dale et al., 2006). Volumetric resonance imaging (MRI) studies also support these features, as does the immune-mediated aetiological hypothesis of a larger caudate, putamen and pallidus in PANDAS patients in comparison with healthy children (Giedd et al., 2000).

As far as the immune-mediated disorders hypothesis is concerned, a single study has demonstrated an improvement of obsessive–compulsive symptoms after plasmapheresis or intravenous immunoglobulin treatment (Perlmutter et al., 1999).

In these disorders, antineural antibodies have been studied by several methods: immunofluorescent antibody staining in human basal ganglia, indirect immunofluorescence in rat striatum, enzyme–immunosorbent assays on human post-mortem basal ganglia tissue, and Western blotting techniques. Western blot allows the presence of specific antibodies against the brain fraction to be investigated where serum is exposed, and it is the best procedure for determining their molecular weight. The reproducibility of the studies described is unclear, and certain discrepancies in their methods and data make them unreliable: for example, differences in brain regions studied, differences in tissue conditions, different and always low serum dilutions (some of them 1:10 or 1:25, and never higher than 1:300) and differences in the analysis of the results (comparison of specific bands between cases and controls or discriminant analyses that show different antibody patterns in subjects and controls).

The aim of the present study using immunohistochemistry and immunoblotting techniques was to detect the presence of specific antineural antibodies in children with OCD or TS, some of whom met criteria for PANDAS, compared with healthy children. Special attention was paid to the methodology and a high serum dilution was used to minimize non-specific binding.

2. Material and methods

The study sample included 32 prepubertal-onset OCD children and adolescents (15 male, 17 female), 21 TS children and adolescents (18 male, 3 female) and 19 normal children (10 male, 9 female), all of them aged between 9 and 17 years. The diagnosis of OCD and TS was made according to DSM-IV criteria (First et al., 1997), and the diagnosis of PANDAS was made in those who met the criteria proposed by Swedo et al. (1998). All patients were seen at the Child Psychiatry Department of the Hospital Clinic in Barcelona, while control subjects were recruited from the community and had no personal history of PANDAS, SC, tics or OCD. The sample was recruited during the period 2001–2003. The procedures were approved by the institution's Ethics Committee and written informed consent was obtained from all parents of subjects under study.

The mean age (±SD) of the three groups was as follows: OCD group, 13 ± 2.9 years; TS group, 12.1 ± 1.9 years; and healthy patients group, 12.5 ± 2.9 years. These differences were not significant ($p = 0.07$). Eight patients met criteria for PANDAS, three of them from the OCD group (2 males, 1 female) and five from the TS group (5 males). Tic severity was assessed using the Yale Global Tic Severity Scale (YGTSS). This instrument has separate scales for motor and vocal tics (Leckman et al., 1989). For obsessive–compulsive symptoms the Children's Yale-Brown obsessive–compulsive scale (CY-BOCS) (Goodman et al., 1989) was used. The CY-BOCS mean ± SD score for OCD patients was 27.5 ± 5.6 (range 14–36), while the mean YGTSS score in TS patients was 41.05 ± 17 (range 20–70). Antistreptolysin-O titres were determined using the standard haemagglutination procedure in all subjects. ASLO titres above 200 U/ml. were defined as positive.

Anti-basal ganglia antibodies were tested by immunohistochemistry on frozen sections of putamen from a normal subject using an avidin–biotin immunoperoxidase