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Differential binding of antibodies in PANDAS patients to cholinergic interneurons in the striatum

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ABSTRACT

Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus, or PANDAS, is a syndrome of acute childhood onset of obsessive-compulsive disorder and other neuropsychiatric symptoms in the aftermath of an infection with Group A beta-hemolytic *Streptococcus* (GABHS). Its pathophysiology remains unclear. PANDAS has been proposed to result from cross-reactivity of antibodies raised against GABHS with brain antigens, but the targets of these antibodies are unclear and may be heterogeneous. We developed an *in vivo* assay in mice to characterize the cellular targets of antibodies in serum from individuals with PANDAS. We focus on striatal interneurons, which have been implicated in the pathogenesis of tic disorders. Sera from children with well-characterized PANDAS (n = 5) from a previously described clinical trial (NCT01281969), and matched controls, were infused into the striatum of mice; antibody binding to interneurons was characterized using immunofluorescence and confocal microscopy. Antibodies from children with PANDAS bound to ~80% of cholinergic interneurons, significantly higher than the <50% binding seen with matched healthy controls. There was no elevated binding to two different populations of GABAergic interneurons (PV and nNOS-positive), confirming the specificity of this phenomenon. Elevated binding to cholinergic interneurons resolved in parallel with symptom improvement after treatment with intravenous immunoglobulin. Antibody-mediated dysregulation of striatal cholinergic interneurons may be a locus of pathology in PANDAS. Future clarification of the functional consequences of this specific binding may identify new opportunities for intervention in children with this condition.

1. Introduction

Obsessive-compulsive disorder (OCD) and tic disorders often first appear in childhood (Regier et al., 1990; Scahill et al., 2001; Taylor, 2011). In a minority of pediatric OCD cases, onset is unusually abrupt and is accompanied by a range of comparably severe associated neuropsychiatric symptoms. This syndrome has been named Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) (Chang et al., 2015; Swedo et al., 2012). In some instances, this abrupt onset is seen with or after resolution of an infectious illness, suggesting an immune-mediated pathogenesis (Allen et al., 1995). Temporal association with infection by group A beta-hemolytic *Streptococcus* infection (GABHS, or *Streptococcus pyogenes*) has been noted with particular frequency; this association has been termed Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus, or PANDAS (Swedo et al., 1998; Williams and Swedo, 2015; Swedo and Williams, 2017).

By analogy with the pathophysiology of Sydenham's chorea (SC), a neuropsychiatric disorder that also occurs following GABHS infection, it was proposed that infection in susceptible children triggers an autoimmune reaction through molecular mimicry, a process in which host antibodies directed against *Streptococcus pyogenes* cross-react with human proteins (Swedo et al., 2012;

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Williams and Swedo, 2015; Carapetis et al., 2016; Kirvan et al., 2003, 2006). In SC, for example, antibodies from patients have been found to cross-react both against neuronal lysoganglioside and streptococcal N-acetyl-beta-D-glucosamine (Kirvan et al., 2003). Other antibody targets have been described in SC, including against the dopamine D2 receptor (Ben-Pazi et al., 2013; Dale et al., 2012). Numerous studies have sought to better characterize the PANDAS clinical subgroup, clarify the associated pathophysiology, and identify the brain targets of the autoantibodies (Williams and Swedo, 2015). Studies in animals have confirmed the ability of anti-Streptococcal antibodies to produce neural and behavioral abnormalities, further justifying the pursuit of antibody targets that may explain pathogenesis (Brimberg et al., 2012; Lotan et al., 2014; Yaddanapudi et al., 2010; Spinello et al., 2016; Macri et al., 2013). Despite this progress, the PANDAS diagnosis remains somewhat controversial, and its pathophysiology remains to be clearly elucidated (Swedo and Williams, 2017).

Based on the hypothesized autoimmune etiology, a variety of immunomodulatory therapies have been investigated in children with PANDAS (Williams and Swedo, 2015). An early controlled study indicated efficacy of both plasmapheresis and intravenous immunoglobulin (IVIG), compared to placebo (Perlmutter et al., 1999). Subsequent clinical experience has continued to suggest benefit from these approaches in some cases (Kovacevic et al., 2015). A recent two-site study, performed at Yale and the National Institute of Mental Health, identified children with PANDAS by particularly stringent criteria and treated them with IVIG or placebo (NCT01281969). While IVIG did not separate from placebo during the blinded phase, response rates were robust after the administration of open-label IVIG, which all participants were offered if their symptoms remained severe after completion of the double-blind phase (Williams et al., 2016).

Functional and structural abnormalities of the cortico-basal ganglia circuitry have been described in both OCD and tic disorders and are central to most current thinking about their pathophysiology (Graybiel and Rauch, 2000; Menzies et al., 2008; Leckman et al., 2010; Williams et al., 2013). Pathological abnormalities in the striatum have been also reported in PANDAS. Giedd and colleagues (Giedd et al., 1996, 2000) found enlarged striatal volume in patients with PANDAS, similar to that seen in those with acute Sydenham's chorea. Striatal abnormalities have been reported to resolve in conjunction with symptoms, either after plasmapheresis (Giedd et al., 1996) or spontaneously (Elia et al., 2005). More recently, inflammation of the striatum has been reported in PANDAS and Tourette syndrome patients, as measured by positron emission tomography using a marker of microglial activation (Kumar et al., 2015).

The hypothesis that PANDAS derives from molecular mimicry implies the presence of antibodies in PANDAS patients that cross-react with brain antigens, as has been documented in Sydenham's chorea (Kirvan et al., 2003). Indeed, such reactivity has been documented *ex vivo* (Singer et al., 2004). Reactivity of antibodies from individuals with PANDAS against several neuronal proteins, including tubulin, lysoganglioside, and dopamine receptors, has been reported, as has antibody-mediated activation of CaM kinase II; these findings have not been consistent across all studies, perhaps due to etiological heterogeneity (Kirvan et al., 2006; Cox et al., 2013, 2015; Morris-Berry et al., 2013; Singer et al., 2015).

The current investigation draws upon recent evidence implicating striatal interneuronal abnormalities in the pathophysiology of tic disorders. *Post mortem* analyses have identified reduced density of specific populations of striatal interneurons in patients with Tourette syndrome; these include cholinergic interneurons (CINs) and GABAergic interneurons expressing the markers parvalbumin and nNOS (Kalanithi et al., 2005; Kataoka et al., 2010; Lennington et al., 2016). Our laboratory has shown that recapitulation of these post-mortem findings in mice, using experimental depletion of cholinergic or parvalbumin-expressing interneurons in the dorsal striatum, produces tic-like phenomenology (Xu et al., 2015, 2016; Rapanelli et al., 2017). This suggests that striatal interneuronal dysfunction may play a causal role in tic pathophysiology. The contribution of interneuronal pathology to OCD is less clear.

To date, no studies have investigated reactivity of antibodies from individuals with PANDAS with epitopes present on interneurons. We investigated interneuron targets of PANDAS antibody reactivity using an *in vivo* model. We infused serum from children with PANDAS into the striatum of mice and characterized the cellular targets of serum antibodies using double immunofluorescence with a panel of cell-specific markers. Samples were drawn from the treatment trial described above, NCT01281969; cases matched particularly stringent clinical criteria for PANDAS (not just PANS), including acute onset of OCD symptoms, presence of characteristic associated symptoms, and documented GABHS infection (Williams et al., 2016). Individuals who responded clinically to IVIG treatment were selected for analysis, as responders may be most likely to have antibody-mediated pathophysiology. We report the first evidence for interneuron-reactive antibodies in the pathogenesis of PANDAS.

2. Methods and materials

2.1. Samples from patients diagnosed with PANDAS

Sera from children with PANDAS were obtained from a recent IVIG trial, NCT01281969 (Williams et al., 2016). This randomized, double-blind trial investigated the efficacy of IVIG on PANDAS symptoms. Subjects in the clinical trial were required to meet all diagnostic criteria for PANDAS, including a positive test for beta hemolytic *Streptococcus*, and to have moderate to severe OCD symptoms, as assessed by the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS (Scahill et al., 1997). Ratings were performed by trained clinicians, blinded to treatment status (Kirvan et al., 2003).

For the pilot study (Supplementary Fig. 2), three children with PANDAS were selected from this cohort, without applying further selection criteria. To enhance the probability of identifying subjects with antibody-mediated pathophysiology in the main experiment (Figs. 1–3), we selected subjects who had a positive clinical response to IVIG, defined as a >35% decrease in the Y-BOCS score (see Fig. 4A). One subject from the pilot experiment met these additional criteria and was included in the main experiment (subject B in the pilot, #3 in the main experiment). Two subjects in the main experiment received IVIG during the blinded treatment phase; two received placebo during the blinded phase and IVIG during the subsequent open-label phase, 6 weeks later. The fifth subject received open-label IVIG infusion and follow-up on the same schedule as the others after being excluded from the trial due to anxiety associated with a lumbar puncture. Serum samples used in this analysis were obtained at baseline and at 12 weeks (time points 1 and 3 in the clinical trial; see Fig. 4A). The second time point was thus either 6 (2 subjects) or 12 weeks (3 subjects) after IVIG infusion. Of the five subjects in the main experiment, two had mild tics at baseline; three had no tics.

Control subjects for the pilot experiment were drawn from healthy control samples collected at Yale. For the main experiment, well-screened healthy subjects matched to the PANDAS sample for age and gender, also evaluated at the NIMH and stored under equivalent conditions and for equivalent time, were used as controls (see Table 1). None of these healthy controls had clinically significant OCD or tic symptoms.

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