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Behavioral and neural effects of intra-striatal infusion of anti-streptococcal antibodies in rats

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ABSTRACT

Group A β-hemolytic streptococcal (GAS) infection is associated with a spectrum of neuropsychiatric disorders. The leading hypothesis regarding this association proposes that a GAS infection induces the production of auto-antibodies, which cross-react with neuronal determinants in the brain through the process of molecular mimicry. We have recently shown that exposure of rats to GAS antigen leads to the production of anti-neuronal antibodies concomitant with the development of behavioral alterations. The present study tested the causal role of the antibodies by assessing the behavior of naïve rats following passive transfer of purified antibodies from GAS-exposed rats. Immunoglobulin G (IgG) purified from the sera of GAS-exposed rats was infused directly into the striatum of naïve rats over a 21-day period. Their behavior in the induced-grooming, marble burying, food manipulation and beam walking assays was compared to that of naïve rats infused with IgG purified from adjuvant-exposed rats as well as of naïve rats. The pattern of *in vivo* antibody deposition in rat brain was evaluated using immunofluorescence and colocalization. Infusion of IgG from GAS-exposed rats to naïve rats led to behavioral and motor alterations partially mimicking those seen in GAS-exposed rats. IgG from GAS-exposed rats reacted with D1 and D2 dopamine receptors and 5HT-2A and 5HT-2C serotonin receptors *in vitro*. *In vivo*, IgG deposits in the striatum of infused rats colocalized with specific brain proteins such as dopamine receptors, the serotonin transporter and other neuronal proteins. Our results demonstrate the potential pathogenic role of autoantibodies produced following exposure to GAS in the induction of behavioral and motor alterations, and support a causal role for autoantibodies in GAS-related neuropsychiatric disorders.

1. Introduction

Group A β-hemolytic streptococcal (GAS) infection is associated with a spectrum of neurological and neuropsychiatric disorders, including Sydenham's chorea (SC), pediatric autoimmune neuropsychiatric disorders associated with streptococcus (PANDAS), obsessive–compulsive disorder (OCD), and Tourette's syndrome (TS) (Dale, 2005; Husby et al., 1976; Peterson et al., 2000; Swedo et al., 1998, 1994; Taranta and Stollerman, 1956). SC is the classical post-streptococcal neurological disorder occurring weeks to months after GAS infection, and is characterized by involuntary movements and neuropsychiatric disturbances, including obsessive–compulsive symptoms, tics, and emotional lability (Marques-Dias et al., 1997; Swedo et al., 1993).

The leading hypothesis regarding the relationship between GAS infection and neuropsychiatric disorders proposes that a GAS infection induces the production of autoantibodies, which cross-react with neuronal determinants in the brain (especially in the basal ganglia, prefrontal cortex and thalamus) through the process of molecular mimicry (Bonthius and Karacay, 2003; Bronze and Dale, 1993; Church et al., 2002; Cunningham, 2000, 2012; Husby et al., 1976; Kirvan et al., 2003; Pavone et al., 2004; Swedo et al., 1994).

Five criteria should be met to establish a disorder as an autoantibody-mediated autoimmune disorder: (A) presence of autoantibodies in the serum or cerebrospinal fluid (CSF); (B) a therapeutic effect of plasma exchange; (C) presence of antibodies

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at the tissue involved in the disorder pathogenesis; (D) induction of symptoms by immunizing with the antigen in an animal model; and (E) induction of symptoms by passive transfer of antibodies to animals (Archelos and Hartung, 2000). Several studies reported the presence of autoantibodies in the sera and CSF of GAS-related neuropsychiatric disorders patients (Bronze and Dale, 1993; Church et al., 2002; Gause et al., 2009; Husby et al., 1976; Kotby et al., 1998; Singer et al., 1998), and plasma exchange treatment has been reported to alleviate symptoms in these patients (Garvey et al., 2005; Perlmutter et al., 1999), fulfilling criteria A and B. We (Brimberg et al., 2012) and others (Hoffman et al., 2004) have found that exposure of rodents to GAS antigen leads to behavioral alterations, fulfilling Criterion D. Others (Ben-Pazi et al., 2012; Doyle et al., 2012; Hallett et al., 2000; Loiselle et al., 2004; Singer et al., 2005; Taylor et al., 2002; Yaddanapudi et al., 2010) have attempted to demonstrate that passive transfer of Immunoglobulin G (IgG) alters behavior (Criterion E). Some have been successful (Doyle et al., 2012; Hallett et al., 2000; Taylor et al., 2002; Yaddanapudi et al., 2010) while others have not (Ben-Pazi et al., 2012; Loiselle et al., 2004; Singer et al., 2005). The aim of the present study was to extend our previous findings (Brimberg et al., 2012) and test whether intra-striatal passive transfer of antibodies to animals would lead to the induction of a behavioral syndrome similar to those reported (Criterion E).

We have recently shown that exposure of male Lewis rats to GAS antigen led to a syndrome which resembles behavioral, pharmacological, immunological and neural characteristics of GAS-related neuropsychiatric disorders (Brimberg et al., 2012). Behaviorally, GAS-exposed rats showed increased compulsive-like behavior and motor disturbances, similar to the neuropsychiatric symptoms found in GAS-related neuropsychiatric disorders. Moreover, the abnormal behaviors in GAS-exposed rats were attenuated by pharmacological treatments used to treat the corresponding symptoms in human patients (i.e., a selective serotonin reuptake inhibitor (SSRI) and a D2 blocker, respectively). Immunologically, IgG was found in the striatum, prefrontal cortex (PFC) and thalamus of GAS-exposed rats (Brimberg et al., 2012), corresponding to the brain regions implicated in GAS-related neuropsychiatric disorders (Barsottini et al., 2002; Citak et al., 2004; Dilenge et al., 1999; Giedd et al., 2000; Huyser et al., 2009). IgG in sera obtained from GAS-exposed rats demonstrated strong immunoreactivity with D1 and D2 dopamine receptors and activated calcium/calmodulin-dependent protein kinase II signaling, as has previously been found for IgG in sera obtained from SC and PANDAS patients (Kirvan et al., 2003, 2006). In addition, we have found alterations in dopamine and glutamate levels in the medial frontal cortex and basal ganglia of GAS-exposed rats (Brimberg et al., 2012), further supporting a functional effect of the autoantibodies.

The aim of the present study was to directly test the role of the autoantibodies in inducing disease-like symptoms and to further test the involvement of the dopaminergic and the serotonergic systems in the induction of behavioral alterations. To this end, IgG purified from the sera of GAS-exposed rats and from adjuvant-exposed rats was infused directly into the striatum of naïve rats (GAS-I and Control-I rats, respectively) and the behavior of infused rats as well as of naïve rats was assessed (for more details see Fig. 1). Our results show that infusion of IgG from GAS-exposed rats leads to behavioral and motor alterations partially mimicking those seen in GAS-exposed rats. In addition, we show for the first time that IgG from GAS-exposed rats reacts with 5HT-2A and 5HT-2C serotonin receptors *in vitro*. *In vivo*, IgG deposits in the striatum of infused rats colocalized with specific brain proteins such as dopamine receptors, the serotonin transporter and other neuronal proteins.

Materials and methods

Animals

Male Lewis rats (Harlan, Jerusalem, Israel), 5 weeks old, were housed in groups of 2–3 per cage under a reversed 12-h light–dark cycle (lights on at 1900–0700 h) with ad libitum food and water. Rats were weighed twice a week. All experimental protocols were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Tel-Aviv University.

GAS-exposure

Streptococcus pyogenes

M protein type 18 (Manfraedo) was obtained from Dr. Allon Moses (Hadassah University Medical Center, Jerusalem, Israel), and grown as previously described (Brimberg et al., 2012). In short, streptococci were grown in 400 ml Todd-Hewitt broth (HyLab, Rehovot, Israel) overnight at 37 °C with rocking at 250 rpm. The next morning, the cells were collected by centrifugation at 5000 rpm for 15 min at 4 °C. The cell pellets (in ~1.5 g) were stored frozen at −20 °C until used.

Mutanolysin-extracted GAS antigen

A whole cell digest of M type 18 *Streptococcus pyogenes* was prepared as previously described (Brimberg et al., 2012). In short, cell pellets were suspended in phosphate-buffered saline (PBS) containing mutanolysin (Sigma–Aldrich, Rehovot, Israel). Following incubation at 37 °C for 2 h with rocking, the digest was further disrupted by sonication (Microson ultrasonic cell disruptor, Plainveiw, NY). The insoluble material was removed by centrifugation at 12,000 rpm (~25,000*g*) for 30 min at 4 °C. Protein concentration in the supernatant was determined using the Coomasie-Plus Bradford reagent (PIERCE) according to the supplier recommendations. The supernatant was dialyzed extensively against water (10,000 MWCO, Sigma–Aldrich, Rehovot, Israel) then lyophilized and the powder was stored at −70 °C.

Exposure of donor rats to GAS antigen

The exposure protocol followed Brimberg et al. (2012). Twenty eight rats were handled for 2 min daily for 4 days before the beginning of the exposure protocol. The first exposure was done at 5 weeks of age. Before each injection, rats were lightly anaesthetized with Isoflorene (VetMarket, Petach Tikva, Israel). Each rat in the GAS group was immunized subcutaneously with 200 µl of 1:1 emulsion of PBS containing 1.2 mg of the GAS antigen and Complete Freund's adjuvant (CFA, Sigma–Aldrich, Rehovot, Israel) supplemented with 4 mg/ml of heat-killed mycobacteria H37RA (Difco Laboratories, Detroit, MI). In order to increase the permeability of the blood brain barrier (Linthicum et al., 1982) rats have received an intraperitoneal injection of 1010 heat-killed *Bordetella pertussis* (Bioport, Lansing, MI, USA) as an additional adjuvant. Two boosts were introduced two and four weeks following the first exposure. Each rat was boosted with 200 µl 1:1 emulsion of incomplete Freund's adjuvant (IFA, Sigma–Aldrich): PBS containing 1.2 mg of the GAS antigen. Control animals were injected with PBS and adjuvants only. Behavioral testing began when the rats were 11 weeks old.

Preparation of pooled GAS and control donor IgG

Rats were euthanized and blood was collected. After clotting and centrifugation, serum was collected and stored at −70 °C. Protein L resin (Genscript, USA) was packed in a polypropylene column (1 ml) and equilibrated with 10 ml of wash buffer (20 mM Na_2HPO_4, 0.15 M NaCl, pH 8.0). Sera samples were filtered by pass-