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IMMUNOLOGY OF NEURODEVELOPMENTAL DISORDERS

Heat shock protein 90 antibodies in autism

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Autism is a complex condition of multifactorial origin, including possible genetic, infectious, and immunological factors. Autism shares features with autoimmune disorders, including genetic susceptibility, association with viral infection, immunologic dysfunction and difference in gender prevalence. Abnormalities of cellular, humoral and innate immunity have been associated with autism. Autoantibodies to a variety of CNS and neuronal targets—including myelin basic protein,[1] frontal cortex,[2] 5HT1A receptors[3] and cerebellar neurofilament[4]—have been detected in autistic subjects. Antineuronal autoantibodies have also been observed in patients with other disorders characterized by repetitive behaviors, including autism, Tourette's syndrome (TS),[5] attention deficit/hyperactivity disorder (ADHD),[6] and obsessive-compulsive disorder (OCD).[7] CNS infection in early life may trigger autoantibodies that cross-react with CNS antigens, leading to disorders with compulsive motor dysfunction, with age at initial infection determining the ultimate phenotype. It has been hypothesized that autism may conceivably be associated with in utero or early postnatal infection, whereas PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Steptococcal Infections) and the Sydenham's chorea sequelae of rheumatic fever may be associated with infection later in life.

Heat shock proteins (hsps) are ubiquitous in human cells, and are critical in the maintenance of proper protein synthesis and function.[8] These proteins may serve as targets for autoimmune insult,[9] with elevated levels of hsp90, a 90-kDa member of the hsp family, and antibodies to hsp90, identified in non-psychiatric illnesses, including systemic lupus erythematosus.[10] Levels of hsp90 are elevated in lupus patients with neuropsychiatric disease, with changes in protein level highly sensitive for disease activity,[11] and in individuals with schizophrenia.[12] We became interested in hsp90 based on our serendipitous observation of a 90 kDa molecular weight protein in Western blots of the serum of autistic individuals with compulsive symptomatology that were being analyzed for a different study. We aimed to determine whether a subset of autistic individuals had elevated levels of autoantibodies to hsp90 compared to other study populations.

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Table 1 Hsp90 serum antibody levels. Mann–Whitney *P* values for autism *vs* other groups

Study group	*n*	*Mean*	*SD*	*P*
Autism	21	7.504	7.200	–
Normal controls	61	3.527	2.109	0.023
Autoimmune illness	35	2.968	1.955	<0.001

We analyzed levels of serum autoantibody to hsp90 in 21 autistic subjects, 61 normal controls, and 35 subjects with an autoimmune disorder—either scarlet fever, rheumatic fever, or acute glomerulonephritis. The Mann–Whitney U-test was used to compare hsp90 serum antibody levels across test populations. *P* values arrived at using the Mann–Whitney U-test to compare autistic subjects with the other study populations are listed in Table 1.

The autistic subjects had significantly elevated levels of antibodies to hsp90 compared to both normal subjects ($P = 0.023$) and those with autoimmune disorders ($P < 0.001$). Further analysis of the specific autoimmune disorders showed that autistic subjects had significantly higher antibody levels than individuals with scarlet fever ($P = 0.002$) or rheumatic fever ($P = 0.006$), but not glomerulonephritis.

Figure 1 is a scattergraph displaying antibody levels

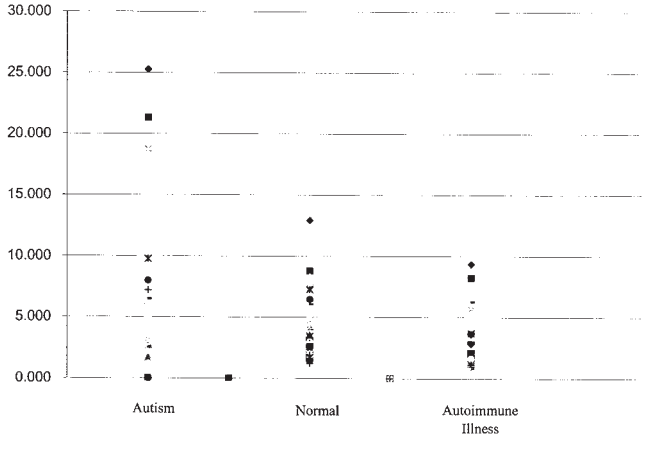

Figure 1 Scattergraph of serum antibody stimulatory index scores. (a) Autism *vs* normals; $P = 0.023$ (Mann–Whitney U-test). (b) Autism *vs* autoimmune illness; $P < 0.001$ (Mann–Whitney U-test).

for autistic subjects, normal controls, and subjects with autoimmune disorders. This figure illustrates the existence of a subset of autistic patients with elevated levels of hsp90 autoantibody not seen in the other study populations. Four of 21 autistic individuals (19%) had stimulatory indices greater than 15, while no subjects in either control group had levels this high. This subset of autistic individuals may represent a unique patient population that largely accounts for the significant differences seen between the autistic and control study groups.

These results support a possible autoimmune pathogenesis for a subset of autism cases. Autistic individuals had a significantly higher level of autoantibodies to hsp90 than did normal controls. A significant difference was also seen between autism and non-psychiatric autoimmune disorders, supportive of a specific role for these antibodies in autistic pathophysiology.

Our findings join those of other studies that have detected elevated levels of anti-CNS or antineuronal antibodies in the sera of autistic subjects, leading to the suggestion that an autoimmune pathogenesis may account for some subset of autistic patients. Higher autoantibody levels in autistic patients of the type reported here, combined with the range of immune abnormalities seen in the autistic population and the model for autoimmune dyskinesia provided by Group A Beta Hemolytic Strep (GABHS) sequelae, suggest a role for autoimmunity in the pathogenesis of a subset of autism. Autoimmune factors are doubtless one part of the story; only a minority of infected children will ultimately develop the type of autoimmune insult leading to autism or related movement disorders, and thus predisposing genetic or environmental factors may play a decisive role. Research has identified a B lymphocyte antigen, D8/17, which has been suggested as a susceptibility marker for rheumatic fever and the childhood onset of autoimmune-mediated disorders with repetitive movement aspects.[13]

Identification of a distinct subset of autistic individuals with a unique pathophysiology may translate into better clinical care and medical outcomes for these patients. In the future, we will need to better characterize these individuals in numerous domains, including other aspects of the immune system (ie, the D8/17 marker), differences in clinical features (ie, repetitive behaviors), the course of illness (ie, waxing and waning), etc. While these patients in theory may benefit from immunomodulatory therapy such as intravenous immunoglobulin with plasmaphoresis, at this time there is no evidence to support such treatment for autistic patients.[14]

One hundred and seventeen subjects, recruited by the Seaver Autism Research Center of The Mount Sinai Medical Center and by Rockefeller University, gave written informed consent to participate in our studies in accordance with the policies of the appropriate Institutional Review Boards. Psychiatric screening of autistic subjects ($n = 21$) was performed by experienced psychiatrists and research nurses using structured interviews and age-appropriate standardized rating scales. Normal controls ($n = 61$) were free from present, past and family history (first-degree relatives) of psychiatric and autoimmune illnesses. Individuals with autoimmune disorders ($n = 35$) were examined by experienced physicians and diagnosed according to accepted medical definitions of their conditions. Of the autoimmune group, 10 subjects had scarlet fever, 10 had rheumatic fever, and 15 had acute glomerulonephritis. Autoimmune individuals did not have any psychiatric conditions or repetitive behaviors. All three study populations consisted of children and adolescents who were of similar ages. Autistic individuals were drawn from a practice in the United States, while normal controls and autoimmune subjects were drawn from a practice in Mexico.

Venous blood samples were obtained from all subjects enrolled in the study, and serum was isolated from each sample utilizing standard procedures. Enzyme-linked immunosorbent assays (ELISA) procedures for the presence and quantification of hsp90 antibody in serum were run on each sample. Hsp90 protein purified from HeLa cells (StressGen Biotechnologies Corp, Victoria, BC, Canada) was diluted 1:100 in 0.1 M $Na_2CO_3/NaHCO_3$ pH 9.6 antigen dilution buffer and 100 μl of this dilution was used to coat each ELISA well overnight at 4ºC. ELISA plates were washed the next day with 0.05% Tween 20 in phosphate buffered saline (PBS) using a Wellwash 4 Mk 2 apparatus (Labsystems, Helsinki, Finland). Wells were filled with 200 μl of 1% bovine serum albumen (BSA) in PBS and left at room temperature for 3 h. Subsequently, subject serum was diluted 1:100 in 0.05 Tween 20 in PBS and 100 μl of serum solution were placed in each well (in triplicate for each subject). ELISA plates were left overnight at 4ºC. The next day the plates were washed as described above. Subsequently a 1:1000 dilution of alkaline phosphatase-conjugated goat affinity purified antibody to human IgG Fc in 0.05 Tween 20 in PBS was prepared and 100 μl of solution were placed in each well. Plates were covered and incubated for 3 h at 37ºC in a laboratory incubator. After incubation the plates were washed as described above. Subsequently 100 μl of *p*-nitrophenylphosphate (p-NPP) mixture (15 mg tablet p-NPP per 5 ml 0.05 M $NaHCO_3/Na_2CO_3$ 0.1 mM $MgCl_2$ pH 9.8) were added to each well. Readings of reactivity as measured by 405 nm wavelength were taken by uQuant ELISA reader. Rat monoclonal hsp90 antibody (StressGen Biotechnologies Corp) was used to confirm that hsp90 protein adhered to the ELISA plates. All ELISA assays were conducted under identical conditions and time parameters. A 'stimulatory index', defined as the absolute reactivity of a serum sample divided by the control background reactivity of that sample's ELISA plate, was used to standardize results precisely across ELISA plates.

The Mann–Whitney U-test, a conservative, non-parametric measure of association, was used to compare stimulatory indices of autistic subjects to the stimulatory indices of other study populations. A non-parametric measure was used due to the non-normal distribution of samples. Statistical calculations were

performed using SPSS software (SPSS Inc, Chicago, IL, USA).

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1 Singh VKA *et al*. *Brain Behav Immun* 1993; **7**: 97–103.
2 Plioplys AV *et al*. *Neuropediatrics* 1989; **20**: 92–102.
3 Todd RD, Ciaranello RD. *Neurobiology* 1985; **82**: 612–616.
4 Plioplys AV *et al*. *Neurology* 1989; **39**(suppl 1): 187.
5 Singer HS *et al*. *Biol Psychiatry* 1999; **46**: 775–780.
6 Peterson BS *et al*. *Arch Gen Psychiatry* 2000; **57**: 364–372.
7 Kiessling LS *et al*. *J Dev Behav Pediatr* 1994; **15**: 421–425.
8 Ohtsuka K, Suzuki T. *Brain Res Bull* 2000; **53**: 141–146.
9 Lamb JR, Young DB. *Mol Biol Med* 1990; **7**: 311–321.
10 Latchman DS, Isenberg DA. *Autoimmunity* 1994; **19**: 211–218.
11 Dhillon VB *et al*. *Q J Med* 1994; **87**: 215–222.
12 Kilidireas K *et al*. *Lancet* 1992; **340**: 569–572.
13 Swedo SE *et al*. *Am J Psychiatry* 1997; **154**: 110–112.
14 DelGiudice-Asch G *et al*. *J Autism Dev Disord* 1999; **29**: 157–160.