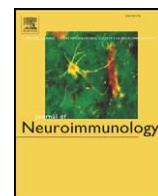




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Immune allergic response in Asperger syndrome

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ABSTRACT

Asperger's syndrome is a subgroup of autism characterized by social deficits without language delay, and high cognitive performance. The biological nature of autism is still unknown but there are controversial evidence associating an immune imbalance and autism. Clinical findings, including atopic family history, serum IgE levels as well as cutaneous tests showed that incidence of atopy was higher in the Asperger group compared to the healthy controls. These findings suggest that atopy is frequent in this subgroup of autism implying that allergic inflammation might be an important feature in Asperger syndrome.

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1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopment disorder characterized by impaired social interaction, verbal and nonverbal communication deficits and restricted or stereotyped patterns of behavior. Recently, the prevalence of the whole spectrum has increased markedly and it is estimated that affects approximately 60–70:10,000 with a biased male-to-female ratio of three or four to one. Alternatively, in the Asperger syndrome the prevalence of 6:10,000 also shows a raising tendency (Fombonne, 2009). Genetic, environmental, neurological, and immunological factors are involved in its pathogenesis, but the etiology of the disorder is unknown (Korvatska et al., 2002; Baron-Cohen and Belmonte, 2005). Many studies have reported neurological abnormalities found in the cerebellum, cerebral cortex and amygdala in autistic patients, but these studies did not advance our understanding about autism any further (Courchesne et al., 2004; Kirsch et al., 2005; Amaral et al., 2008).

Besides neurological abnormalities, inflammatory pathways activated due to immune dysfunction, have been proposed as possible mechanisms implicated in the pathogenesis of autism. Many immunologic studies have described imbalances in immune and inflammatory processes in peripheral blood of autistic patients (Ashwood et al., 2006). Previous reports have found an increased frequency of circulating anti-

brain autoantibodies (Todd and Ciaranello, 1985; Wills et al., 2009) and myelin basic protein (Singh et al., 1993). T-cell dysfunction with increased activation of both Th2 and Th1 arms of the adaptive immune response, with Th2 predominance (Gupta et al., 1998; Molloy et al., 2006) and the antibodies reactive to the Central Nervous System proteins (Zimmerman et al., 2007) has been described. In addition to immunological findings, active neuroinflammation has been described in the cerebral cortex and cerebellum of postmortem brain tissue, characterized by marked cellular activation of microglial and astroglial cells and altered cytokine production without a clear pattern have also been described (Vargas et al., 2005). All together, these findings suggest that abnormal immune responses may influence neural function and could play a role in the development of autism.

Epidemiological studies have provided evidence for the association of asthma and allergies in families with autistic children (Becker, 2007). However, this view has not been systematically studied and was controversial when family history, skin tests and serum IgE levels were evaluated (Bakkaloglu et al., 2008). Alterations in the pattern obtained in the serum immunoglobulin (Ig) subclass profile, i.e. increased serum IgG2 and IgG4 in children with autism compared with typically developing control children (Croonenberghs et al., 2002; Enstrom et al., 2009) have been demonstrated. Moreover, IgG4 like IgE has been implicated in allergy development (Oxelius, 2008). However, there is no consistent data about allergic inflammation in patients with ASD. A clinical heterogeneity exists within ASD in which subtypes are classified in relation to deficits ranging in severity among patients including individuals that are high functioning classified as Asperger's syndrome (Gaigg and Bowler, 2007). The characterization of distinct groups or clinical

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phenotypes would facilitate studies avoiding discrepancies in the identification of its diverse biological status. The aim of this study was to investigate allergic immunological features in Asperger autistic patients.

2. Materials and methods

2.1. Selection of patients

In the period of January, 2006 to December, 2008 fifteen male Asperger patients were obtained from a total of 206 ASD evaluated in the Neurology Outpatient Clinic of Instituto Fernandes Figueira (IFF). All Asperger patients were excluded if they had epilepsy or morphological alterations evaluated by Computerized Tomography (CT) scans. These patients meet DSM-IV-TR and Filipek's review criteria (American Psychiatry Association et al., 2000; Filipek et al., 1999, respectively) and these parameters were considered in a consent statement from the American Academy of Neurology and Child Neurology Society (Filipek et al., 2000), and also from the American Academy of Pediatrics (Johnson et al., 2007) to define the diagnosis of autism and Asperger syndrome. Cognitive function was evaluated using the WISC-III assessment of IQ (Welchsler, 1991) to separate subgroups of the spectrum (Witwer and Lecavalier, 2008). Asperger's patients with IQ score equal or higher than 70 were classified as high functioning (Filipek et al., 1999; Macintosh and Dissanayake, 2004; Kaland et al., 2008). The age range of Asperger patients varied from 7 to 18 years at the time of sample collection (Table 1). Of the 15 patients, 1 had been previously treated with risperidone for one year, 1 with fluoxetine and 1 with methylphenidate. Patients under psychotropic or other drugs that might interfere with the cutaneous response were excluded. In addition, 15 normal non-atopic controls and 15 atopic age-matched controls obtained from the Pediatric/Adolescent follow-up and Allergy outpatient clinics of IFF, respectively were selected. There were no differences between the three groups in terms of age, sex and socioeconomic status. Asperger's patients were excluded if they were using oral steroids or antihistamines or suffered from any disease that could interfere with immunological status. Ethics committee of IFF has authorized this study and all families gave written and verbal consents for the procedures.

2.2. Clinical evaluation

All autistic children and adolescents enrolled in the present study were Asperger patients with IQ equal or higher than 70. They were evaluated by conventional clinical neurologic examination and formal intelligence assessment using the WISC-III. All Asperger patients,

normal non-atopic control and atopic controls were clinically evaluated to confirm diagnosis of rhinitis, atopic dermatitis or asthma in accordance to the literature, and skin prick tests to allergens were performed employing the same antigens in all groups. Asperger and atopic controls were classified as atopic based on clinical history in relation to rhinitis, atopic dermatitis and asthma symptoms with positive skin test reactivity to allergens.

2.3. IgE measurement and eosinophil count

Serum was collected from Asperger, normal and atopic patients and stored at -20°C . Total IgE levels are represented as IU/mL and were determined by Neselometry assay method using BN-PROSPEC. The estimation of eosinophil percentage was done using the SYSMEX XT1800i.

2.4. Skin prick tests

To confirm atopic status, all Asperger patients, normal non-atopic controls and atopics underwent skin prick testing to *Blomia tropicalis* (*Blo t*) (0.8 mg/mL), *Dermatophagoides pteronyssinus* (*Der p*) (0.68 mg/mL) and *Dermatophagoides farinae* (*Der f*) (0.6 mg/mL, all allergens were obtained from ALK-ABELLÓ, Denmark). Histamine dihydrochloride was used as a positive control and physiologic saline solution as a negative one. Skin prick testing was performed with tests at least 5 cm apart, and wheal size was read after 15 min and a response was recorded as positive if the average diameter of the wheal reached at least 3 mm above negative control for at least one antigen.

2.5. Statistical analysis

Results were analyzed by Prisma software version 5.0 using unpaired Student's *t*-test for comparing variables of the groups. Spearman's correlation was used to analyze relationships between variables with known impact on atopic disease. The Mann–Whitney test was used for between-group comparison. A *p* value of <0.05 was considered to be statistically significant.

3. Results

The sample consisted of 15 male autistic Asperger patients aged 13.3 ± 2.9 years old. Fifteen age-matched normal non-atopic controls aged 12.6 ± 3.1 years old and the same number of an atopic group, as a positive control, aged 10.6 ± 3.1 years old were studied. The mean age was 12 ± 3 years. The characteristics of Asperger patients are shown in Table 1.

The frequency of allergic diseases between the Asperger, normal control and atopic groups is shown in Fig. 1. Initially, positive family history (FH) of atopy in the Asperger patients was verified and the results showed that age was not correlated with atopic disease in the Asperger group ($p = 0.138$). These patients showed a high incidence of allergic symptoms when compared to normal controls. With regard to respiratory allergy in the Asperger group, allergic rhinitis was predominant in 53.3% (8/15) of patients, whereas 2/15 had rhinitis plus dermatitis, one showed coexistence of allergic rhinitis and asthma and one patient suffered just from asthma.

Skin prick tests comparing Asperger and normal controls are shown in Fig. 2. It is possible to observe that there is a higher incidence of positive skin prick tests among Asperger patients compared to normal controls. There was a positive association between cutaneous reactivity to *Blo t*, *Der p* and *Der f* in the Asperger group. The majority of these patients (11/15) had reactivity to *Der p*. Of the 15 Asperger patients recruited, 9/15 had skin prick tests positive to *Blo t* and 10/15 to *Der f* (Table 1). From normal controls only one showed a positive skin test to *Der p* and *Der f*. However, none of the normal controls had

Table 1
Clinical descriptive characteristics and skin prick test of Asperger patients.

Patient	Current age	Current medication	Clinical features	FH to allergy	Cutaneous test		
					<i>Blo t</i>	<i>Der p</i>	<i>Der f</i>
1	7	Risperidone	Rhinitis	Yes	–	+	–
2	15	Fluoxetine	Rhinitis	Yes	+	+	+
3	13	None	Rhinitis	Yes	+	+	+
4	18	None	Rhinitis	No	+	+	+
5	13	None	Rhinitis	Yes	–	–	–
6	12	None	Rhinitis	Yes	+	+	+
7	16	None	Asthma	Yes	+	+	+
8	15	Ritalin	–	Yes	–	–	–
9	9	None	Rhinitis and dermatitis	Yes	–	+	+
10	13	None	Rhinitis	No	+	+	+
11	15	None	Rhinitis	Yes	+	+	+
12	16	None	–	No	–	–	–
13	10	None	Asthma; rhinitis	Yes	+	+	+
14	16	None	–	No	–	–	–
15	12	None	Rhinitis; dermatitis	Yes	+	+	+

Blo t = *Blomia tropicalis*.

Der p = *Dermatophagoides pteronyssinus*.

Der f = *Dermatophagoides farinae*.

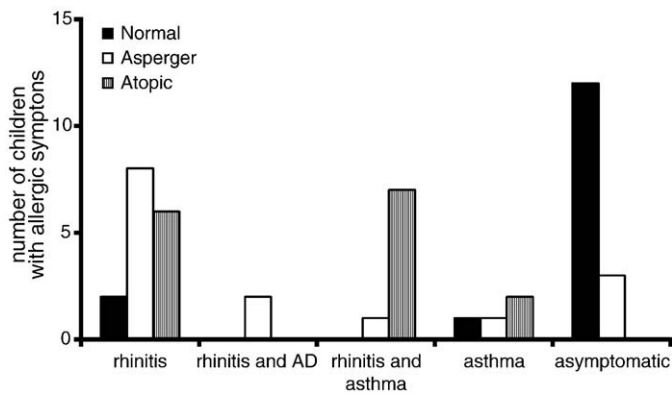


Fig. 1. Clinical manifestations of allergic disease in Asperger, normal and atopic children. AD = atopic dermatitis.

positive skin tests to *Blot*. Eleven Asperger patients had family history of allergic diseases whereas only one control had such a history.

In addition, we evaluated other allergic features including IgE levels and eosinophil count in the Asperger group, healthy controls and atopic patients. Total IgE was elevated in 13 Asperger patients (Fig. 3). The Asperger group had high levels of serum total IgE (802.0 ± 905.5 IU/mL/ $p < 0.0017$) when compared to normal group (156.1 ± 233.4 IU/mL). Similar IgE levels were found in Asperger and atopic patients (841.6 ± 683.5 IU/mL) as shown in Fig. 3. An increase in eosinophils was also observed when Asperger patients were compared to normal controls (Fig. 4, $p < 0.003$). There is no correlation between IgE levels and eosinophil count ($p = 0.437$). However, Spearman's model showed that there is a correlation between number of antigens and eosinophils ($R = 0.85$, $p < 0.000$) in Asperger patients. These results were confirmed by the Mann–Whitney test that showed atopy in the Asperger group is associated with eosinophil count and number of antigens ($p = 0.003$, $p = 0.001$, respectively).

Considering the clinical findings, cutaneous test and clinical history, a higher incidence of atopy was observed in the Asperger group when compared to healthy controls (Fig. 5). In conclusion, autistic patients Asperger in type were atopic with a high predominance of rhinitis.

4. Discussion

Atopic diseases including eczema, asthma and rhinitis are the commonest chronic diseases of childhood resulting in allergic inflammation. The prevalence of atopic disease is reduced in Brazil compared to developed countries (Strachan et al., 1998; Miescher and Vogel, 2002)

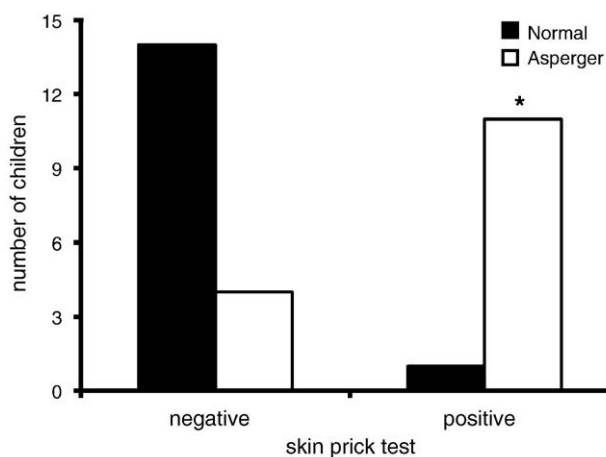


Fig. 2. Number of Asperger children with reactivity to allergenic test compared to healthy controls. * $p < 0.05$ for Asperger versus normal subjects in positive skin prick test.

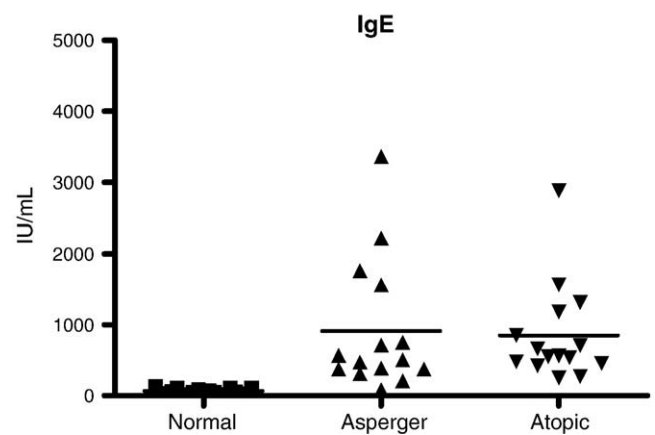


Fig. 3. Scatterplot showing individual differences in serum total IgE levels among Asperger, normal and atopic children. Black lines indicate geometric mean concentrations of IgE. Mean (SEM) in Asperger group was 802.0 ± 905.5 IU/mL significantly different from normal group ($p < 0.001$). Atopic group was different from normal group ($p < 0.001$). No statistical difference was observed between Asperger and atopic group ($p = 0.6582$).

and several studies suggest an association of declining incidence of infections with the rising prevalence of atopic disease (Sublett, 2005). In accordance to this, we found unexpectedly a high incidence of atopy in Asperger patients and a positive family history of atopy as well.

Allergic features in autistic patients were described before based on family history, however, no differences were obtained on IgE levels and skin prick tests between autistic patients and controls (Bakkaloglu et al., 2008). In the present study, we showed that the frequency of atopic dermatitis, asthma and predominantly rhinitis were increased in Asperger patients compared to healthy controls. We verified that total IgE levels associated with eosinophilia were elevated in nearly 90% of patients. It is known that parasitological diseases can interfere with IgE levels and eosinophil count, which could explain these results, however, stool tests for parasitological infections were done showing negative results. The normal controls and Asperger patients were obtained from our Maternal–Child hospital where we could find the groups studied showing the same social and environmental conditions which are factors influencing the allergic response. Therefore, we consider that a homogeneous sample could facilitate the discrimination of biological findings.

Many studies have shown a clinically relevant association of eosinophil count and IgE levels with atopy (reviewed by Miescher and Vogel, 2002). Allergic symptoms and inflammatory markers, including

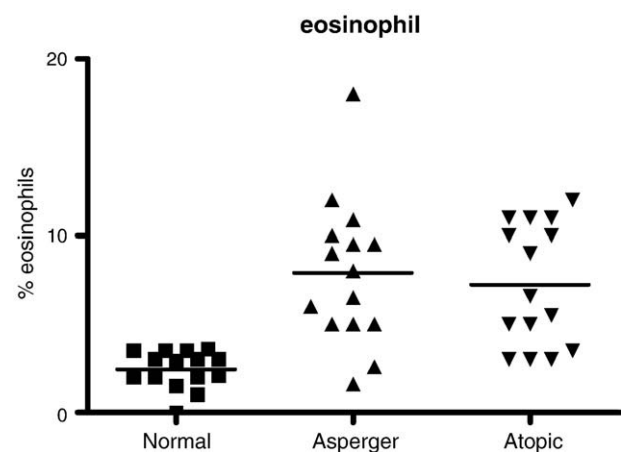


Fig. 4. Scatterplot showing individual differences in eosinophil percentual among Asperger, normal and atopic children. Black lines indicate geometric mean of eosinophil percentual. Significant difference was observed in Asperger group in comparison to normal control ($p < 0.001$). Atopic group was different from normal group ($p < 0.001$). No statistical difference was observed between Asperger and atopic group ($p = 0.9669$).

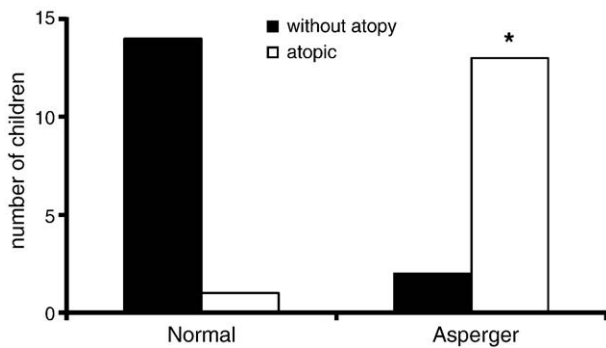


Fig. 5. Incidence of atopy in Asperger group compared to healthy controls. * $p < 0.05$ for atopic Asperger versus atopic normal subjects.

increase in total IgE levels and eosinophil counts, provide evidence in support of atopy. In addition, skin prick test results revealed that Asperger patients were positive to allergens, predominantly *Der p* and *Der f*, associated with respiratory allergy. These allergens were chosen as being predominant in the Brazilian atopic population (Baqueiro et al., 2007). Considering these features 86.6% of Asperger patients were atopic in contrast to less than 7% among age-matched healthy controls. Furthermore, when compared to age-matched atopic patients, the Asperger group showed similar increased levels of IgE and eosinophils. In our analysis, by using Spearman's correlation we could evidence that the number of antigens and eosinophils was associated with atopy development in the Asperger patients sensitized to allergens. Therefore, these data indicate that Asperger patients do have an increased tendency to atopy when compared to the normal population.

Another study published by Jyonouchi et al. (2008) evaluated ASD children and found abnormalities in cytokine production by assessing responses to TLR agonists in peripheral blood mononuclear cells of ASD patients with frequent infections. The authors did not find differences when comparing the incidence of primary immunodeficiency, food allergy non-IgE-mediated and atopy in healthy individuals compared to ASD patients, in the presence or absence of frequent infections. The selected ASD patients were regressive, different from our study that investigated allergic response in an Asperger group. It is unknown whether the inflammatory response is different among subgroups of autistic patients, but this is a possibility that could explain the difference between these two studies.

Many reports have described imbalances in immune and inflammatory responses in autism. Increases in IL-12 and IFN- γ levels have been observed in plasma cytokine analysis studies in autistic children compared to controls (Singh, 1996). On the other hand, others authors have demonstrated increase in IL4 but decrease in IFN- γ in T lymphocytes stimulated *in vitro*, suggesting a Th2 bias (Gupta et al., 1998). Besides that, levels of Th2 cytokines were significantly higher in stimulated peripheral blood mononuclear cells from autistic children compared to controls (Molloy et al., 2006). However, in that particular study, no differences were found in clinical history, allergic symptoms and absolute eosinophil count between autistic patients and controls. Most of the patients were taking single medication or in combination, such as fluoxetine, sertraline and risperidone, however no significant differences were observed between cases taking or not psychotropic medication for any cytokines.

There are conflicting results in the literature that could be attributed to differences in age and confound factors like the use of medication. A number of studies evidenced effects of psychotropic medications such as lithium, benzodiazepines and the atypical antipsychotic clozapine, in increasing cytokine levels (Haack et al., 1999; Pollmacher et al., 2000). However, it is not clear whether the cytokine response is affected by selective serotonin reuptake inhibitors or other atypical antipsychotic medications such as risperidone (Rabkin et al., 1999; Zhang et al., 2004; Basterzi et al., 2005). In the present study only three patients were under

medication. One patient under methylphenidate treatment did not present increase in eosinophil count or IgE levels, despite family history of allergic inflammation, whereas two patients with allergic symptoms, positive skin prick test and high IgE level were under risperidone or fluoxetine treatment. Therefore, in most of our patients, the results obtained could not be attributed to medication.

The interpretation of immune findings in the autism spectrum may be difficult due to the differences among subgroups that include regressive type, autism with mental retardation and Asperger patients. In general the subgroups are not specified and to avoid discrepancies based on phenotypes, in our study we evaluated only Asperger type of autism. The studies on autism and allergy in the medical literature did not determine whether or not the Asperger group is associated with allergic symptoms, or whether the presence of allergy is a characteristic of all autistic subgroups. The present findings may reflect a pattern of response in Asperger patients and implicate that analysis of the allergic response might be important to the approach of these patients. The expectative is that immunological findings could support future approaches to therapy for this alarming neuropsychiatric disease.

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