
Immune function in autistic children

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Background: There have been reports that some children with autistic spectrum disorders have abnormal immune function. However, data in this area remain scarce and conflicting.

Objective: To evaluate the immune function of a series of autistic children in the context of this proposed association.

Methods: We prospectively collected data on 24 autistic children who, between January 1, 1996, and September 30, 1998, were referred unsolicited to an immunology clinic. We examined the clinical history and evaluated immunoglobulin levels; specific antibody titers to diphtheria, tetanus, and *Haemophilus influenzae*; T- + B-cell numbers; T-cell proliferation; and complement studies.

Results: Seven of the 24 children had a history of recurrent infections. Only 2 patients had immunoglobulin levels that were outside the age-adjusted reference ranges, 1 of whom was subsequently diagnosed as having common variable immune deficiency. All the patients had normal in vitro T-cell function and complement study results, and only 2 of 24 patients had subtle derangements in T-cell numbers. Elevated levels of IgE were found in 5 patients, which correlated with a clinical history of atopy. Low diphtheria or tetanus antibody levels were found in 12 patients, but in 11 of these, vaccination status was not up-to-date.

Conclusions: Most of the autistic children studied had normal immune function, suggesting that routine immunologic investigation is unlikely to be of benefit in most autistic children and should be considered only when there is a history suggestive of recurrent infections.

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INTRODUCTION

Autism is a pervasive developmental disorder characterized by impairment in social interactions, deficits in verbal and nonverbal communication, and an unusual, repetitive, or severely limited range of activities and interests.¹ The prevalence of all autism spectrum disorders, including autistic disorder, pervasive developmental disorder not otherwise specified, and Asperger syndrome, is currently estimated to be approximately 60 per 10,000 population.² It occurs 4 times more frequently in males, and the prevalence is relatively stable across cultures and nations. The symptoms usually appear before the age of 36 months and continue throughout life, with a guarded prognosis.

Autism is a disorder of uncertain etiology and limited treatment options. In a small percentage of patients, autism is observed in association with other medical conditions, such as congenital rubella, birth anoxia, encephalitis, untreated phenylketonuria, tuberculous sclerosis, and fragile X syndrome.^{2,3} The symptoms seem to be manifestations of a physical dysfunction in the central nervous system, the exact nature of which has not yet been determined. Theories of etiology have centered on genetic abnormalities, infectious causes, pathologic responses to vaccination, abnormal neurochemistry, and gastrointestinal disturbances, among others. However, neither a coherent anatomic or pathophysiologic

theory of autism nor a biological diagnostic test has been developed. Understandably, parents of autistic children often seek answers and remedies wherever they can, including outside the traditional health care system.

Among other theories, immune dysregulation has been postulated. Many immunologic studies have found abnormalities in subsets of autistic patients, suggesting that immune system dysregulation could either cause or be associated with the neurobehavioral disturbances of autism. These studies have centered around immune deficits,^{4–11} autoimmune phenomena,^{12–21} and sequelae of viral exposure^{22–26} and have yielded a variety of findings and theories about the relationship between the neurologic and immune systems in this disorder. Results have been contradictory, however, without consistently replicated findings or a unified theory to account for the various findings.

Reports of immune deficiencies have prompted questions about immunologic treatments for this condition. Gupta et al¹¹ treated a subsample of their cohort with intravenous immunoglobulin (IVIG) in an unblinded manner with reportedly good results, but no systematic behavioral or psychometric assessments were performed, and it was unclear how the subsample was chosen. Two subsequent studies^{27,28} of IVIG suggested a lack of response, but samples were still small, studies were uncontrolled, and assessments were unblinded, although standardized measures of behavior were used in 1 study. Therefore, some researchers have cautioned against the use of costly, inconvenient, and as-yet unproven treatments in this population outside of research protocols.²⁷

It remains unclear whether there indeed are specific immune abnormalities or less specific alterations in immune

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variables associated with autistic spectrum disorders. To further investigate the potential association of immune abnormalities and autistic disorder, we prospectively studied an unsolicited cohort of autistic children to determine the spectrum of immune function in this population.

MATERIALS AND METHODS

Patients

We prospectively collected data on 24 children (20 boys and 4 girls) aged 3 to 17 years (mean age, 5 years) who were referred to an immunology clinic at the Montreal Children's Hospital by pediatricians, neurologists, and the Quebec Society for Autism with a diagnosis of autism or pervasive developmental disorder. The patients were seen in the clinic between January 1, 1996, and September 30, 1998. All the patients underwent the same immune evaluation, and their immune function was compared with standard laboratory reference ranges.

Immunologic Laboratory Assessment

Immunoglobulin levels, including serum IgG, IgM, and IgA levels, were measured using laser nephelometry (Behring Inc, Laval, Quebec). Serum IgE levels were measured using enzyme-linked immunosorbent assay (ELISA) (Sanofi Pasteur, Montreal, Quebec). Specific antibody titers to diphtheria and tetanus were measured using ELISA (SCIDMEDX; Whitaker Biosource, Montreal), as previously reported.²⁹ *Haemophilus influenzae* antibody levels were also measured using ELISA (Binding Site, Birmingham, England).

Enumeration of T- and B-lymphocyte subsets was performed using flow cytometry (FACScan; Becton-Dickinson, Mississauga, Ontario). Lymphocytes ($1 \times 10^5/\text{mL}$) were stained using the following antibodies: anti-CD3, anti-CD19, anti-CD4, anti-CD8, and anti-CD16 for natural killer (NK) cells. All antibodies were purchased from Becton-Dickinson. Appropriate isotype controls were used to determine the gating for analysis. Levels of C3 and C4 complement were measured using nephelometry (Behring Inc). Total hemolytic complement and total alternate pathway function were measured using hemolytic assays with sensitized sheep and rabbit erythrocytes, respectively. Serum samples for these tests were separated and frozen immediately at -80°C until they were processed according to the method of Harbeck and Giclas.³⁰

T-Cell Proliferation

T-cell proliferation was performed from peripheral blood samples. Three to 5 mL of blood was procured in a heparinized tube. The lymphocytes were separated using Ficoll-Paque (Pharmacia, Uppsala, Sweden) density centrifugation (2,000 rpm for 30 minutes) and were washed 3 times before plating. Cells were incubated with mitogens (phytohemagglutinin, concanavalin A, and pokeweed mitogen) (Sigma, Toronto, Ontario) for 72 hours in 96-well plates in a humidified incubator at 37°C , with 5% carbon dioxide. After 66 hours, $1 \mu\text{Ci}$ per well of tritiated thymidine (ICN, Laval) was added, and the cells were incubated for 6 hours. The cells

were harvested using water lysis (PHD Cell Harvester, Cambridge, MA) and thymidine incorporation quantified by liquid scintillation counting (LKB, Gaithersburg, MD). Results are expressed as counts per minute, and patients were compared with controls, in whom studies were performed in parallel.

RESULTS

Patient Characteristics

The characteristics and vaccination status of the 24 referred children are given in Table 1. All the patients had been diagnosed as having autistic disorder or pervasive developmental disorder not otherwise specified by a community pediatrician, a developmental pediatrician, or a neurologist. A history of frequent infections was reported by the parents for 7 patients (29%) based on open questions, including the following: "Does your child have more infections than other peers in his or her class or day care?" "Does your child require antibiotics more than 3 times a year?" "Does your child get recurrent infections other than colds?" The identified patients had recurrent fevers, otitis media, sinusitis, pharyngitis, or other upper respiratory tract infections. None of them had a history of severe infection, such as meningitis, bacteremia, or other invasive infections. Pneumonia was also infrequent. Five patients (21%) were up-to-date with the recommended vaccinations at the time of the visit. One patient (4%) had been vaccinated only until the age of 12 months, 17 patients (71%) had been vaccinated until the age

Table 1. Characteristics of 24 Patients Referred for Evaluation*

Patient No./sex/age, y	Frequent infections	Vaccination status	Atopy
1/M/6	+	Until 18 mo	—
2/M/5	+	Until 18 mo	—
3/M/6	+	Until 18 mo	+
4/F/3	—	Until 18 mo	+
5/M/3½	—	Until 18 mo	+
6/M/7	+	Up-to-date	—
7/M/4	—	Until 18 mo	+
8/M/4½	—	Up-to-date	+
9/M/4	—	Until 18 mo	—
10/F/3½	—	Until 18 mo	+
11/M/3½	—	Until 18 mo	—
12/M/4½	—	Up-to-date	—
13/M/5½	—	Until 18 mo	+
14/F/3	—	Until 12 mo	—
15/M/4½	+	Until 18 mo	—
16/M/3	—	Until 18 mo	—
17/M/7½	+	Up-to-date	—
18/M/6	+	Up-to-date	+
19/M/3	—	Until 18 mo	—
20/M/3½ (twin)	—	Until 18 mo	+
21/M/3½ (twin)	—	Until 18 mo	+
22/M/3	—	Until 18 mo	—
23/M/17	—	Until 4 y	+
24/F/2½	—	Until 18 mo	—

* Plus sign indicates positive; minus sign, negative.

of 18 months, and 1 teenager (4%) was vaccinated until the age of 4 years and was due for his adult diphtheria and tetanus booster vaccination. Most patients had not continued the standard vaccination schedule after the diagnosis of autism or pervasive developmental disorder. In terms of atopy, 11 patients (46%) had a personal history of allergic rhinitis, asthma, atopic dermatitis, or food allergy.

Humoral Immunity

All the patients had a normal number of total B cells (CD19) (see the "T-Lymphocyte Studies" subsection) (Table 2). The mean $\pm$ SD values of IgG, IgM, and IgA for the group were 10.72 ± 3.39 g/L, 1.37 ± 0.55 g/L, and 1.05 ± 0.53 g/L, respectively. The subgroup with frequent infections was comparable, with mean $\pm$ SD levels of IgG, IgM, and IgA of 11.35 ± 3.94 g/L, 1.32 ± 0.56 g/L, and 1.08 ± 0.45 g/L, respectively. Of the 24 patients, most ($n = 22$) had immunoglobulin levels within the reference range for age. Only 2 patients had immunoglobulin levels that were outside the age-adjusted reference range (Table 3). Patient 2, who had a history of recurrent fevers, sinusitis, and otitis, had low levels of IgG (4.96 g/L) and IgM (0.47 g/L) for his age. He also had an inadequate response to booster vaccines. Based on this history, he was diagnosed as having common variable immune deficiency (CVID) and was treated with monthly IVIG.

Patient 13 had elevated IgG (17.9 g/L), borderline high IgM (2.20 g/L), and normal IgA (1.45 g/L) levels. He did not experience recurrent infections. This patient was subsequently diagnosed as having Landau-Kleffner syndrome, an inflammatory neurologic disease.

We found that 11 patients (46%) had a personal history of atopy; 5 of them had elevated levels of IgE (ie, >240 μ g/L). Patient 3 had asthma and food allergies to peanuts and tree nuts, patient 4 was allergic to soy, patient 5 had atopic dermatitis, patient 7 had allergic rhinitis and extensive atopic dermatitis, and patient 13 had atopic dermatitis as an infant. The food allergies were confirmed by skin testing. The mean value of IgE was 212.58 μ g/L in the entire group and 378.64 μ g/L in the atopic subgroup.

We examined levels of specific antibodies to the vaccines commonly used in this population—diphtheria, tetanus, and *H influenzae*. Twenty-one (87.55%) of 24 patients had protective antibody levels to all 3 antigens (>0.2 IU/mL for diphtheria and tetanus and >0.15 μ g/mL for *H influenzae*) (Table 4). Three patients had specific antibody levels below the protective level for 1 of the 3 antigens but had protective levels of other antibodies. All these patients had nonprotective levels of antibody to diphtheria. Patient 2 agreed to a booster vaccination and did not have an adequate response,

Table 2. T- and B-Cell Phenotypes Performed Using Flow Cytometry*

Patient No.	CD3 (T cells)		CD19 (B cells)		CD4 (helper cells)		CD8 (cytotoxic cells)		CD4-CD8	CD16 (NK cells)	
	$\times 10^9$ /L	%	$\times 10^9$ /L	%	$\times 10^9$ /L	%	$\times 10^9$ /L	%		$\times 10^9$ /L	%
1	5.040	72	1.050	15	2.380	34	1.890	27	1.3	0.560	8
2	2.953	69	0.514	12	1.669	39	1.156	27	1.4	0.642	15
3	3.250	65	0.550	11	1.900	38	1.300	26	1.5	0.850	17
4	3.550	71	1.250	25	2.100	42	1.450	29	1.4	0.250	5
5	1.904	68	0.644	23	1.176	42	0.616	22	1.9	0.196	7
6	1.920	60	0.672	21	1.248	39	0.672	21	1.9	0.576	18
7	2.772	66	0.840	20	0.924	22	1.134	27	0.8	0.336	8
8	2.108	68	0.496	16	1.333	43	0.775	25	1.7	0.558	18
9	2.320	58	0.480	12	1.480	37	1.160	29	1.3	0.560	14
10	2.040	68	0.750	25	1.050	35	0.660	22	1.6	0.210	7
11	3.976	56	1.775	25	2.485	35	1.136	16	2.2	1.420	20
12	3.256	74	0.704	16	1.760	40	1.276	29	1.4	0.352	8
13	1.350†	45†	0.630	21	0.450†	15†	0.900	30	0.5†	0.630	21
14	2.479	67	0.740	20	1.369	37	0.888	24	1.5	0.296	8
15	1.633	71	0.437	19	1.173	51	0.391	17	3.0	0.161	7
16	5.568	58	2.688	28	2.976	31	2.688	28	1.1	0.672	7
17	2.210	65	0.748	22	1.462	43	0.714	21	2.0	0.442	13
18	1.988	71	0.420	15	1.204	43	0.756	27	1.6	0.252	9
19	4.307	73	0.708	12	2.124	36	1.947	33	1.1	0.649	11
20	2.240	64	0.665	19	1.400	40	0.840	24	1.7	0.315	9
21	2.646	63	0.966	23	1.596	38	0.966	23	1.7	0.546	13
22	1.495	65	0.644	28	0.989	43	0.529	23	1.9	0.115	5
23	1.494	83	0.216	12	0.864	48	0.522	29	1.7	0.108	6
24	2.967	69	1.161	27	1.849	43	0.903	21	2.0	0.258	6

Abbreviation: NK, natural killer.

* Values reflect absolute values and percentages of T and B lymphocytes in peripheral blood samples.

† Abnormal values.

Table 3. Immunoglobulin Levels and Age-Adjusted Reference Ranges

Patient No.	Age, y	IgG (reference range), g/L	IgM (reference range), g/L	IgA (reference range), g/L	IgE, μ g/L*
1	6	13.70 (6.87–18.79)	2.12 (0.54–2.02)	1.85 (0.65–3.68)	58
2	5	4.96† (6.61–17.49)	0.47† (0.54–2.02)	0.79 (0.59–3.28)	14
3	6	13.20 (6.87–18.79)	1.83 (0.54–2.02)	0.58 (0.65–3.68)	384†
4	3	10.00 (6–15.75)	0.94 (0.66–2.57)	0.83 (0.40–2.35)	672†
5	3	11.00 (6–15.75)	1.63 (0.66–2.57)	0.71 (0.40–2.35)	639†
6	7	11.50 (7.13–19.05)	1.48 (0.54–2.02)	1.52 (0.69–3.93)	58
7	4	16.20 (6.26–16.53)	2.01 (0.51–2.01)	2.10 (0.46–2.88)	1,446†
8	4	7.94 (6.26–16.53)	1.39 (0.51–2.01)	0.70 (0.46–2.88)	109
9	4	14.80 (6.26–16.53)	1.32 (0.51–2.01)	1.31 (0.46–2.88)	119
10	3	10.80 (6–15.75)	2.46 (0.66–2.57)	0.87 (0.40–2.35)	20
11	3	12.70 (6–15.75)	1.02 (0.66–2.57)	0.93 (0.40–2.35)	38
12	4	7.71 (6.26–16.53)	0.89 (0.51–2.01)	1.00 (0.46–2.88)	21
13	5	17.90† (6.61–17.49)	2.20† (0.54–2.02)	1.45 (0.59–3.28)	438†
14	3	9.04 (6–15.75)	1.70 (0.66–2.57)	1.00 (0.40–2.35)	136
15	4	9.80 (6.26–16.53)	1.31 (0.51–2.01)	1.13 (0.46–2.88)	45
16	3	9.47 (6–15.75)	1.42 (0.66–2.57)	0.40 (0.40–2.35)	180
17	7	8.99 (7.13–19.05)	0.84 (0.54–2.02)	0.84 (0.69–3.93)	161
18	6	17.30 (6.87–18.79)	1.19 (0.54–2.02)	0.87 (0.65–3.68)	60
19	3	6.77 (6–15.75)	0.64 (0.66–2.57)	0.70 (0.40–2.35)	12
20	3	6.98 (6–15.75)	1.03 (0.66–2.57)	0.71 (0.40–2.35)	67
21	3	7.94 (6–15.75)	1.51 (0.66–2.57)	0.98 (0.40–2.35)	149
22	3	8.18 (6–15.75)	0.80 (0.66–2.57)	0.79 (0.40–2.35)	30
23	17	9.83 (8–18.01)	0.57 (0.54–2.22)	2.62 (1.13–5.63)	181
24	2	10.60	2.03	0.49	65

* The reference range is 0 to 240 μ g/L for all ages.

† The immunoglobulin level was outside the age-adjusted reference range.

which led to the diagnosis of CVID. The other patients with low or borderline (0.2 IU/mL) levels of anti-diphtheria antibody refused booster vaccinations. All these patients had protective levels of antibody to tetanus. We also found 4 patients (17%) with borderline levels of antibody to tetanus, but again none accepted booster vaccination. All these patients had protective levels of anti-diphtheria antibody. All the patients tested had adequate levels of antibodies to *H influenzae*.

In the subgroup of 7 patients with frequent infections, 5 patients (71%) had normal antibody levels to the 3 antigens, 1 patient was diagnosed as having CVID (as previously described), and patient 15 had borderline antibody levels to diphtheria, in the context of not being up-to-date with vaccinations, and otherwise normal findings on immune workup. Only 5 patients had followed the normal recommendations for vaccination schedule. The remainder of the patients were due for booster vaccinations.

T-Lymphocyte Studies

All the patients had normal NK cell counts, and 23 patients (96%) had normal CD3, CD4, and CD8 counts (Table 2). Patient 13 (diagnosed as having Landau-Kleffner syndrome) had a low percentage of total T cells but a normal absolute count. He also had a decreased CD4 count and an inverse CD4-CD8 ratio. None of the subgroup of patients with frequent infections had T-cell subset abnormalities.

T-Cell Proliferation

Lymphocyte stimulation was normal for 21 (95%) of the 22 patients who underwent the test (Table 5). Patient 19 had a viral illness at the time of the testing and exhibited mildly decreased proliferation of T cells compared with same-day controls, but no other immune abnormality was found. A repeat of the study was suggested to his parents but was refused because the rest of his immune workup was normal.

Complement Studies

Complement studies were performed in 21 of the 24 patients. Levels of C3 and C4 were within the reference range in all patients. Total hemolytic complement levels were normal in all but 1 patient, in whom it was mildly decreased at 18 U (reference range, 25–43 U). Total alternate pathway function was also normal in all but 1 patient, in whom it was slightly decreased at 21 U (reference range, 28–144 U). Neither of these 2 patients had any other immune abnormality or a history of frequent infections.

DISCUSSION

In this study of 24 children with autism spectrum disorders, we found only 1 patient with a well-defined immune deficiency, patient 2, who had a history of recurrent infections, abnormal levels of immunoglobulins, and an inadequate response to booster vaccination, all of which led to a diagnosis of CVID. This child was treated successfully with IVIG for 6

Table 4. Levels of Specific Antibodies to Diphtheria, Tetanus, and *Haemophilus influenzae**

Patient No.	Diphtheria, IU/mL	Tetanus, IU/mL	<i>H influenzae</i> , µg/mL
1	6.0	6.0	1.3
2	0.1†	0.3	NA
3	1.0	0.9	1.0
4	0.1†	0.6	1.4
5	4.0	4.0	1.6
6	0.3	2.0	1.5
7	0.2‡	0.3	5.3
8	6.3	9.0	2.0
9	0.2‡	3.0	1.1
10	0.1†	0.6	2.0
11	0.5	0.2‡	1.8
12	0.2‡	6.0	4.1
13	0.8	0.2‡	NA
14	1.0	0.2‡	6.4
15	0.2‡	0.5	1.2
16	2.0	0.4	2.2
17	0.5	2.0	6.0
18	5.0	8.0	NA
19	2.0	0.3	0.4
20	0.6	0.5	0.4
21	0.2‡	0.3	0.4
22	10.0	2.0	8.0
23	0.6	0.2‡	NA
24	0.4	0.8	1.9

Abbreviation: NA, not available.

* Protective levels were greater than or equal to 0.2 IU/mL for diphtheria and tetanus and greater than or equal to 0.15 µg/mL for *H influenzae*.

† Nonprotective level.

‡ Borderline protective level.

years; his subsequent clinical course was notable for decreased episodes of infection, but his behavior and communication remained highly impaired. Patient 13 was found to have elevated immunoglobulin levels and abnormal T-cell enumeration. He was subsequently diagnosed as having Landau-Kleffner syndrome, an inflammatory neurologic disease, and was treated with oral prednisone at a different hospital. No other patient was found to have significant immunologic abnormalities. Considering that this sample consists of children who were referred to an immunology clinic, the paucity of abnormal findings is even more striking. In this way, our findings differ significantly from most of the published results of immunologic assessments of autistic children.

Reported immune system deficits in autistic individuals have included inadequate antibody responses to rubella vaccine,⁵ depressed T-lymphocyte responsiveness to mitogens,^{4,6} decreased numbers of T lymphocytes with altered ratios of T-helper to T-suppressor cells,⁶ decreased NK cell activity,⁷ alteration in the suppressor-inducer T cells,³¹ and decreased numbers of B cells and T-helper cells.¹⁰ However, none of these findings have been consistently replicated, and there

have also been findings of no immune deficits and of increased immune response.^{27,32-35} One group¹¹ found a large number of subtle, unexplained immune abnormalities in a group of autistic children, including decreased levels of serum IgG and IgA, increased levels of serum IgM, and increases and decreases in IgE and in T- + B-cell enumeration. Other studies have reported IgA deficiency⁹ but also findings of elevated IgG levels³³ and normal immunoglobulin levels.³⁶

Reasons for the differences between these studies and ours are not clear at this time. The differences are possibly the result of publication bias, whereby negative studies have not been written up or published. There is also the potential effect of differing methods and materials used in immunologic investigations and differences in ages and sources of the samples across studies. The small samples and the vast differences in what is being measured and how, as well as the wide variation in findings across studies, make it difficult to draw any conclusions from this body of literature at this time.

A history of atopy was reported in 11 (46%) of our 24 patients, which is higher than in the general population. This could be partly due to a referral bias; parents who visit allergy and immunology clinics on a voluntary basis are more likely to be referred because their children are also allergic. Associated elevated IgE levels (>240 µg/L) were found in 5 children and were related to a clinical history of allergic rhinitis, asthma, atopic dermatitis, or food allergy. Results of skin testing confirmed food allergy in the 3 individuals whose history suggested IgE-mediated disease. The level of IgE is similar to that found by Renzoni et al³⁷ in their autistic and control groups, although they found increased IgE levels in patients with and without complaints of allergic symptoms. In their sample, 67% of autistic children had positive histories of allergic symptoms. Because neither our study nor that by Renzoni et al³⁷ was designed to strictly characterize the allergic status of the patient, these results serve only as an indicator that this topic deserves further study. Our group is currently participating in a more detailed study that should address these questions.

With respect to IVIG, although some researchers have suggested IVIG as a useful treatment,^{11,12} their studies had substantial limitations. The few "improved" patients did not have a particular immune profile that could be identified in advance of treatment. Given the lack of evidence of immune dysfunction in our sample and because IVIG is an expensive treatment with inherent risks and potential issues of shortage, we suggest, as have others,^{27,28,38} that it should only be used in cases in which there is a proven indication or in a blinded research protocol.

Although the present study aimed to examine the possibility of immune system abnormalities in this sample, we did not seek to address the question of autoimmunity in autism and so did not investigate levels of autoantibodies in these children. Neither was it our aim to explore etiologic theories related to measles-mumps-rubella vaccination. However, where our results touch on such theories is that they provide

Table 5. Lymphocyte Proliferation in Patient and Controls*

	Patients (n = 22)	Controls
Unstimulated	432 (175–939)	429 (179–732)
Phytohemagglutinin	128,686 (48,539–274,713)	115,436 (23,254–227,438)
Concanavalin A	98,452 (31,698–214,898)	85,348 (27,824–178,167)
Pokeweed mitogen	39,764 (5,141–167,508)	45,023 (8,193–124,713)

* Data are given as mean (range) counts per minute of tritium.

evidence of a current trend away from vaccination in some families of autistic children.

Specific antibody levels to diphtheria, tetanus, *H influenzae*, or pneumococcus and booster responses to vaccines are the standard way to verify the qualitative production of antibodies. Of the 24 patients in our study, 12 were found to have nonprotective or borderline protective titers to either diphtheria or tetanus. This lack of protective levels of antibodies in our sample is likely the direct result of not having received booster vaccinations because 19 (79%) of the 24 children were not up-to-date with their vaccination schedules. Furthermore, only 1 of these patients accepted a booster vaccination (the patient subsequently diagnosed as having CVID). All other parents refused any further vaccination of their children, afraid that vaccination could have been a causal factor in their child's developmental disorder. In addition, children in the study ideally would have evaluation of polysaccharide antibody production by response to a pneumococcal vaccine. Again, the inability to convince parents to vaccinate children in this cohort mitigated against this important part of the immune evaluation.

This situation reflects a new wariness about vaccination among parents, encouraged in great part by the work of Wakefield et al,^{23,24,39} who authored articles that suggest an association of gastrointestinal disease (ileal-lymphoid hyperplasia), developmental regression, and measles-mumps-rubella vaccine. Despite significant methodological concerns^{40–43} and the existence of large epidemiologic studies that dispute this association,^{40,44–50} the suggestion of this relationship has attracted much public attention and has made parents reluctant to vaccinate their children.⁵¹ A corresponding decline in vaccination rates has been documented in England, where coverage declined from 92% in 1997 to 84% in 2001.⁴⁰ Our data indicate a similar effect. Given that the World Health Organization estimated the number of measles cases for 1998 to be 30 million, with 880,000 measles-related deaths preventable with adequate vaccination, it is crucial that parents receive up-to-date information highlighting the safety and importance of vaccination.⁵²

In summary, we did not find a pattern of abnormal immunologic function in the autistic children we studied. The importance of settling the question of vaccination safety in the minds of their parents was highlighted by our findings. However, the low numbers of other abnormalities found among our patients suggest that autistic children are unlikely to have detectable immune abnormalities found on routine screening and that the clinical history would be a better

predictor of the clinical utility of an immunologic evaluation. In light of these findings, immunologic investigation should be recommended in autistic children only when indicated by clinical history or for research purposes.

Given the varied results of immunologic studies in autistic children, it seems important that future studies aim to evaluate the extent to which previous findings are replicable and to explore immunologic variables from a theory-driven perspective so that the findings may contribute more substantially to our growing knowledge in this field. To this end, establishment of large and representative samples, homogeneous with respect to diagnosis, with adequate control groups, and accounting for factors such as age, other illnesses, and medication use, will be important in improving the validity of future findings in this area of burgeoning interest.

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