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Immune Abnormalities in Patients with Autism[1]

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We have begun an investigation on the immune systems of patients with autism in attempt to determine if immune mechanisms are involved in the development of this severe developmental disorder. A study of 31 autistic patients has revealed several immune-system abnormalities, including reduced responsiveness in the lymphocyte blastogenesis assay to phytohemagglutinin, concanavalin A, and pokeweed mitogen; decreased numbers of T lymphocytes; and an altered ratio of helper to suppressor T cells. Immune-system abnormalities may be directly related to underlying biologic processes of autism, or these changes may be an indirect reflection of the actual pathologic mechanism.

INTRODUCTION

Despite increasing research efforts, the cause of autism remains obscure. Within the past few years, however, several workers have found evidence that immune system abnormalities may be associated with autism. Stubbs (1976) observed that some autistic patients have defective antibody responses to rubella vaccine, and in a separate investigation, evidence was found that autistic patients have depressed lymphocyte responsiveness to the T-cell mitogen phytohemagglutinin (Stubbs, Crawford, Burger, &

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Vandenbark, 1977). In addition, Weizman and associates reported that some autistic patients make inappropriate cell-mediated immune response to basic myelin protein, a component of brain myelin (Weizman, Weizman, Szekely, Wijsenbeek, & Livni, 1982). Abnormal immune response to basic myelin protein is known to be associated with multiple sclerosis, a disease now believed by many to have an autoimmune etiology (reviewed by Waksman, 1981).

This article reports findings on the first in a series of studies exploring possible direct or indirect involvement of aberrant immune function in the pathogenesis of autism. The number and function of T and B lymphocytes in autistic patients were investigated and compared to findings in healthy age-matched subjects.

METHOD

Subjects

This study included 31 autistic subjects ranging in age from 3 to 28 years, and 15 healthy subjects who were 3 to 32 years of age. The patients included 24 males and 7 females, with a mean age of 11.0 years. The healthy subjects, faculty and staff members of the Developmental Center for Handicapped Persons and their children, included 10 males and 5 females, with a mean age of 13.2 years. All of the patients had been under the care of a physician since the diagnosis of their disease. Nineteen of the patients satisfied DSM-III criteria used for establishing the diagnosis of autism (termed complete syndrome), and the remainder of the patients satisfied some of these criteria (partial syndrome). Six of the patients, 5 with the complete syndrome and 1 with the partial syndrome, were receiving antiepileptic drugs (AED) at the time of study, including Dilantin, Tegretol, Mysoline, and phenobarbital. Three other patients, all with the complete syndrome, were receiving fenfluramine (as part of an investigation being carried out by Dr. Edward Ritvo and associates), and 1 patient with the partial syndrome also had trisomy 21. All but 7 patients were living at home at the time of their study.

Procedure

Lymphocyte Blastogenesis Assay. The lymphocyte blastogenesis assay was carried out as previously described (Warren, Stembridge, & Gardner, 1985). Briefly, peripheral blood mononuclear cells (PBMC) were obtained

from freshly drawn blood by the use of ficoll-hypaque and suspended in RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) containing penicillin, streptomycin, and 10% autologous plasma or 10% fetal calf serum. Lymphocytic responses to the mitogens phytohemagglutinin (PHA), and to pokeweed mitogen (PWM) (GIBCO, Grand Island, N.Y.) and concanavalin A (con A) (Flow Laboratories, McLean, Va.) were assayed by culturing 10^6 PBMC with various concentrations of mitogens in flat-bottom 96-well tissue culture plates. After the cultures were incubated for 68 h in 5% CO2, tritiated thymidine was added for an additional 4 h. The cells were harvested and assayed with a scintillation counter.

Lymphocyte Enumeration. Total white blood cell numbers and percentage of lymphocytes in the leukocyte population were determined by routine laboratory procedures. Monocyte-depleted PBMC were enumerated with the rosette assay and the complement-dependent cytotoxicity assay. In the cytotoxicity assay, monocyte-depleted PBMC were incubated with OKT4 and OKT8 monoclonal antibodies (Ortho Diagnostic Systems, Raritan, N.J.) and the 7.2 antibody (New England Nuclear, Boston, Mass.) in Microtest II plates for 1 h. They were then incubated in complement and the viable cells determined by trypan blue exclusion. Percent lysis was calculated as lysed cells/total number of lymphocytes counted. Absolute numbers of lymphocyte subsets were calculated using results of the various enumeration procedures.

Statistical Analyses. Data were analyzed with the *t* test and analysis of variance.

RESULTS

Immune function data were analyzed according to three patient groups including patients with the complete syndrome, those with the partial syndrome, and an overlapping group of patients with either the complete or partial syndrome who were receiving AED.

Lymphocyte Blastogenesis Assay. Response of patient PBMC to PHA in concentrations of 1, .5, and .25% are presented in Figure 1. The patients in each group had PBMC that demonstrated severely depressed responses to all three concentrations of PHA, as compared to responses of cells from the healthy subjects ($p < .001$). A significantly depressed response by the PBMC of patients from each group to con A (concentrations of 10, 5, and 2.5 ug/ml) also was observed (Figure 2). Cells of patients from all groups also had significantly reduced responses to PWM (concentrations of .06, .03, and .015) (Figure 3).

Lymphocyte Enumeration. In Table I are presented the results of enumerating the PBMC of the patients. A significantly depressed mean

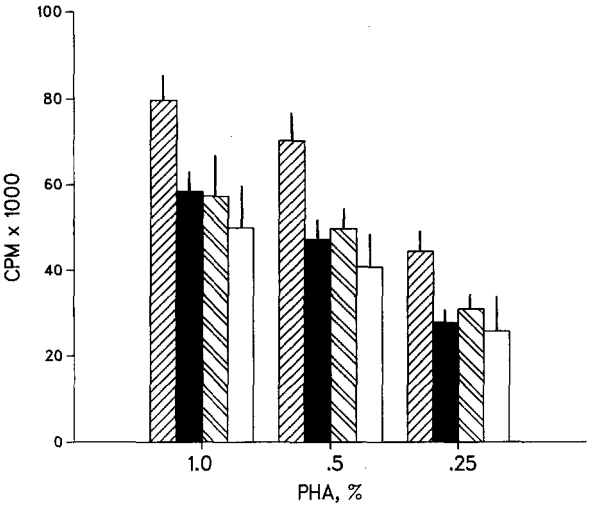

Fig. 1. Phytohemagglutinin-induced incorporation in mean counts/minute (CPM) and standard error of the mean of tritiated thymidine by peripheral blood mononuclear cells from autistic patients expressing the complete syndrome (■), expressing the partial syndrome (▧), receiving antiepileptic drugs (□), and healthy subjects (▨). Incorporations by cells from patients of all groups was significantly depressed ($p < .001$) at each concentration of phytohemagglutinin (PHA).

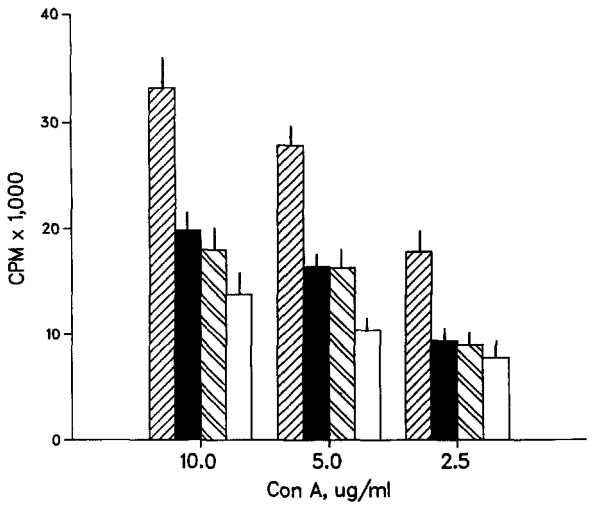

Fig. 2. Concanavalin A-induced incorporation in mean counts/minute (CPM) and standard error of the mean of tritiated thymidine by peripheral blood mononuclear cells from autistic patients expressing the complete syndrome (■), expressing the partial syndrome (▧), receiving antiepileptic drugs, (□), and healthy subjects (▨). Incorporation by cells from patients of all groups was significantly depressed ($p < .001$) at each concentration of concanavalin A (Con A).

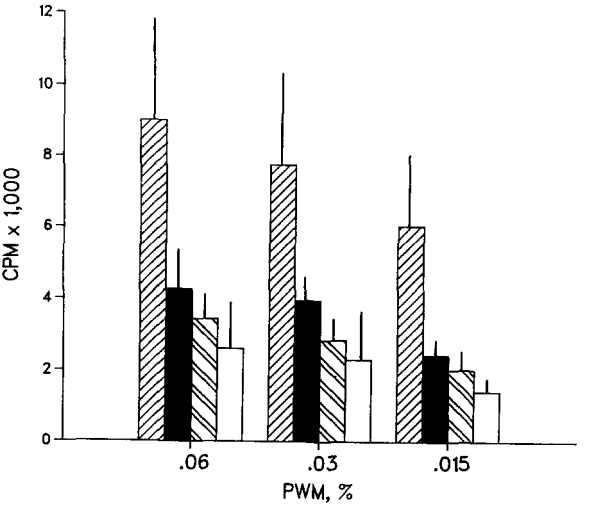

Fig. 3. Pokeweed mitogen-induced incorporation in mean counts/minute (CPM) and standard error of the mean of tritiated thymidine by peripheral blood mononuclear cells from autistic patients expressing the complete syndrome (■), expressing the partial syndrome (▨), receiving antiepileptic drugs (□), and healthy subjects (▨). Incorporation by cells from patients of all groups was significantly depressed ($p < .001$) at each concentration of pokeweed mitogen (PWM).

Table I. Lymphocyte Enumeration

Subjects	*n*	Percent rosette-forming cells[a]	T4:T8 ratio[b]	7.2+[c]
Healthy	15	75.4	2.12	14.8
		7.2[d]	.81	6.0
Patients				
Total	23	61.4[e]	1.62[e]	18.4
		8.1	.59	7.6
With complete syndrome	13	59.6[e]	1.53[e]	18.7
		8.7	.60	6.8
With partial syndrome	10	63.7[e]	1.73[e]	18.0
		7.0	.58	8.8
Patients receiving AED[f]	6	60.3[e]	1.12[e]	19.4
		3.4	.40	12.1

[a]Mean percent of peripheral blood mononuclear cells (PBMC) forming rosettes with sheep red blood cells.
[b]Mean ratio of PBMC reactive with the anti-OKT4 antibody to those reactive with the anti-OKT8 antibody.
[c]Mean percent of PBMC reactive with the anti-7.2 antibody.
[d]1 standard deviation.
[e]Patient values significantly lower than those of the healthy subjects ($p \pm .01$).
[f]Patients currently being treated with antiepileptic drugs.

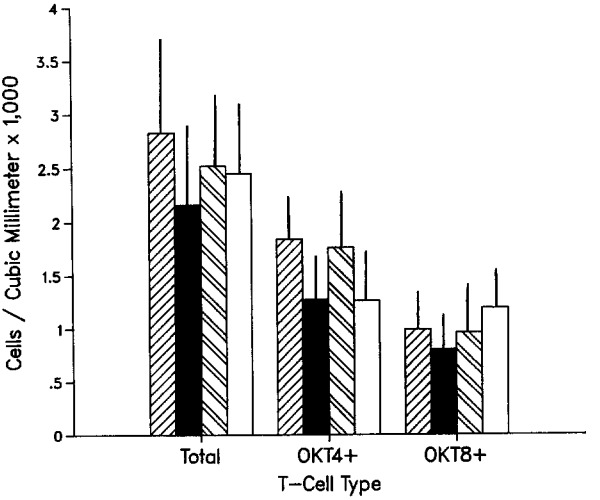

Fig. 4. Mean number/cubic millimeter of blood plus 1 standard deviation of total T cells, T cells expressing the OKT4 marker and T cells expressing the OKT8 marker in autistic patients expressing the complete syndrome (■), expressing the partial syndrome (▨), receiving antiepileptic drugs (□), and healthy subjects (▨). Patients with the complete syndrome had significantly reduced numbers of T cells ($p < .05$) and reduced numbers of OKT4+ cells ($p < .05$).

ratio of OKT4:OKT8 cells was observed in the cell preparations from patients of each of the three groups. In addition, percent rosette-forming cells were significantly lower in the PBMC of patients from each group. In contrast, the proportion of 7.2 positive cells (B lymphocytes) were not significantly different in patients from any of the three groups. In Figure 4 are presented the results of calculating the total number of T cells, OKT4+ cells, and OKT8+ cells. Patients expressing the complete syndrome had significantly reduced numbers of T cells and OKT4+ cells ($p < .05$) but normal numbers of OKT8+ cells. Patients with the partial syndrome and those receiving AED had reduced numbers of total T and OKT4+ cells, but the differences were not significant.

DISCUSSION

The current study confirms an earlier finding (Stubbs et al., 1977) that lymphocytes of patients with autism have defective response to the T-cell mitogen PHA, and indicates that lymphocytes of autistic patients also have significantly reduced responses to the T-cell mitogen con A and the B-cell

mitogen PWM. In addition, the current investigation provides information about the basis of these low mitogenic responses in autistic patients. Monocyte-depleted preparations of patient PBMC demonstrated significantly reduced numbers of total T cells as assessed by the rosette assay. Further, this reduced number of total T cells was probably due to a decrease in the number of T cells expressing the OKT4+ phenotype since the patients had significantly lower numbers of OKT4+ cells but normal numbers of OKT8+ cells. OKT4+ T cells constitute the largest subset of T cells in the blood of healthy subjects, being about twice as frequent as OKT8+ cells, the other major subset of T cells. Since the OKT4+ subset includes cells with a helper/inducer function, our findings suggest that some autistic patients could be deficient in cells with this activity. This suggestion is consistent with our finding that B lymphocytes of the patients had deficient response to PWM, a response that requires the cooperation of helper T cells (Warren, unpublished). However, some cells expressing the OKT4 marker have been reported to have a suppressor cell function instead of a helper function (Thomas et al., 1981). The lack of suppressor cells in the autistic patients may be consistent with an autoimmune process underlying the development of autism. Unfortunately, it is not possible at present to determine the exact T-cell defect in these patients.

Our findings in the autistic patients are quite similar to those observed in a study of patients hopitalized for major depressive disorder. Schleifer and associates reported that a group of such patients had significantly reduced responses to the same three mitogens that were used in our study, had a lower number of T cells, and, in contrast to our finding in autistic patients, had fewer B lymphocytes (Schleifer, Keller, Siris, Davis, & Stein, 1985). Several studies of schizophrenic patients also have found suppressed responses to mitogens, and variously reduced numbers and/or percentages of T and B cells (Coffey, Sullivan, & Rice, 1983; DeLisi, Goodman, Neckers, & Wyatt, 1982; Nyland, Ness, & Lunde, 1980; Zarrabi et al., 1979). However, the findings in the schizophrenic patients are complicated by the fact that they were receiving neuroleptics at the time of their study; neuroleptics have been shown to suppress responses to mitogens. The data in this study were analyzed according to the extent of symptoms exhibited by the patients. Response to mitogens by cells of patients expressing only some of the symptoms of autism were generally quite similar to those observed in patients expressing the complete syndrome; however, the former were not as deficient as the latter in their numbers of total T cells and OKT4+ T cells, percent of rosette-forming cells, and ratio of OKT4:OKT8 cells. If immune-system abnormalities are directly or indirectly involved in the etiology of autism, one might expect patients expressing more symptoms to have greater immune abnormalities. In a separate analysis, cells from patients receiving AED demonstrated levels of mitogen

responsiveness, and ratios of OKT4:OKT8 cells were much lower than those of cells from the patients not receiving AED. However, it is doubtful that the AED alone were responsible for the lower immune values in these patients since a group of concurrently studied patients receiving AED, but who were not autistic, did not have significantly reduced responses to mitogens, and had OKT4:OKT8 ratios that were much higher than the patients receiving AED who were autistic. These latter data are being presented in a separate report.

The implication of immune changes in autistic patients is not clear, but it is possible that they are related to the underlying biologic processes in autism. Several rather speculative possibilities for a relationship between immune changes and the biologic processes of autism exist. Viruses or gestational organisms have been increasingly associated with autism (reviewed by Stubbs et al., 1977). It is possible that a genetic predisposition to relative deficiency in helper T-cell function makes the fetus more susceptible to damage by viruses. Alternatively, viruses may contribute to the immune defect by being present in the fetus at an early stage of immune differentiation and not be recognized at a latter time because of a mechanism of immunologic tolerance.

Elevated blood serotonin concentrations occur in 30–40% of patients with autism (Ritvo et al., 1970). Further, a recent study suggests that lymphocytes from healthy subjects exposed in vitro to high concentrations of serotonin have inhibited responses to PHA (Slauson, Walker, Kristensen, Wang, & DeWeck, 1984). Therefore, it is possible that the higher blood serotonin levels in the autistic patients are inhibitory to the mitogenic responses of their lymphocytes. However, additional studies are needed to investigate this possibility.

Yates (1984) has reviewed recent neuroanatomical findings in autism, relating them to clinical symptomatology. He notes that many of the functional impairments consistently observed in autistic children suggest damage to the left hemisphere of the brain. This concept of left hemispheric damage being associated with autism is interesting in light of recent findings in mice that left-sided brain lesions are associated with impairment of T-cell function. Specifically, Renoux and associates (reviewed by Renoux, 1984) found that mice with surgically induced lesions of the left neocortex had impaired responses to T-cell mitogens, reduced numbers of splenic T-cells, and altered expression of T-cell surface antigens. All of these immune changes seen in the brain-damaged mice were observed in our group of autistic patients, raising the possibility that impaired T-cell function in these patients is a reflection of a brain lesion.

Clearly, additional studies are needed to explore immune system abnormalities in autism. Such investigations may help to elucidate the

pathophysiology of autism and/or the relationship of autism to other disorders associated with immune systems abnormalities such as major depressive disorder.

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