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Abnormal Immune Response to Brain Tissue Antigen in the Syndrome of Autism

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Cell-mediated immune response to human myelin basic protein was studied by the macrophage migration inhibition factor test in 17 autistic patients and a control group of 11 patients suffering from other mental diseases included in the differential diagnosis of the syndrome of autism. Of the 17 autistic patients, 13 demonstrated inhibition of macrophage migration, whereas none of the nonautistic patients showed such a response. The results indicate the existence of a cell-mediated immune response to brain tissue in the syndrome of autism. (Am J Psychiatry 139:1462–1465, 1982)

The term "autism" was introduced into the child psychiatric literature by Kanner (1) in 1943 to characterize disturbances of affective contact in early childhood (onset before the age of three years). Since then, various investigations (2) have been undertaken in an endeavor to categorize and define the etiology of this disease. Some investigators (3–6) proposed an involvement of brainstem dysfunction in autistic children. Damasio and Maurer (7) suggested a neural bilateral dysfunction of the ring of mesolimbic cortex (located in the mesial, frontal, and temporal lobes), the neostriatum, and the anterior and medial thalamic nuclei as a result of perinatal viral infection or genetically determined neurochemical abnormalities. To our knowledge, however, no evidence for diagnosable neuropathological changes in this disease has been found as yet.

Reactivity to human myelin basic protein measured by a migration inhibition factor test has been implicated in the pathophysiology of a number of lesions involving the CNS, e.g., multiple sclerosis (8–10), the Guillain-Barré syndrome (11), the Vogt-Koyanagi-Harada syndrome (12), and in acute disseminated encephalomyelitis (13).

The present study was undertaken to examine the possibility of cell-mediated autoimmune response toward human basic protein in the syndrome of autism. The presence of such a response might point to an accessible brain antigen and therefore strengthen the theory that an organic brain lesion, although neuropathologically undetectable, is involved in the pathogenesis of autism. We employed the migration inhibition factor technique by using the autistic patients' lymphocytes.

METHOD

The subjects of this study were 22 males and 6 females ranging in age from 6 to 22 years. All were studied intensively and evaluated as inpatients in the pediatric and adolescent departments of three

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The authors thank Prof. N. Trainin, Department of Cellular Biology, Weizmann Institute of Science, Rehovot, Israel, for his help in preparing the manuscript.

Israeli psychiatric hospitals. The subjects were divided into two groups.

Subjects

Autistic patients. Seventeen patients (15 males and 2 females) aged 7 to 22 years were diagnosed as suffering from the syndrome of autism according to the criteria suggested by the National Society for Autistic Children (2) and Cohen and associates (14). The criteria included an age at onset of less than 30 months, gross disturbances in social attachment, disturbances in verbal and nonverbal communication, bizarre responses to various aspects of the environment, and severe disturbance of both appropriate affective expression and regulation of anxiety and arousal. The patients' cognitive and adaptive skills as well as their behavioral acts demonstrated the presence of intellectual ability of globally retarded functioning. Motor development was within the normal range, with no evidence of gross dysfunction in motor skills, e.g., walking, running, and fine motor coordination. All the patients were physically healthy and without any evidence indicating diagnosable organic brain syndrome. Evaluations were independently carried out by three of us who are child psychiatrists (A.W., R.W., G.A.S.); complete agreement was required for the establishment of this diagnosis.

Control subjects. Eleven patients (7 males and 4 females) aged 6 to 19 years comprised the control group; they were selected according to the differential diagnosis of the syndrome of autism suggested by the National Society for Autistic Children (2) and Rutter (15). Five of the patients were diagnosed as having childhood schizophrenia on the basis of the presence of definable thought process and content disorders and perceptual distortions as proposed in *DSM-III*, and by Fish and Ritvo (16). Three of the group were mentally retarded based on the following criteria: an IQ score below 70; developmental delays in motor, social, and cognitive areas; and adaptive skills below the normal range, although their social interrelationship and emotional attachments were appropriate to global level of intellectual competence. Patient 1 suffered concomitantly from deafness of unknown origin. One child suffered from emotional deprivation, which was diagnosed according to a history of institutionalization at the age of 3 years and symptoms of failure to thrive, apathy, and withdrawal; he was hospitalized in a psychiatric ward because of disturbances in interpersonal relationships and behavior disorders. One patient, diagnosed as having chronic organic brain syndrome due to encephalitis at the age of 4 years, had symptoms that included a poor memory, attention and concentration deficiencies, disturbances in impulse control, mood lability, and hyperkinesis. One subject suffered from developmental aphasia. The diagnosis was made on the basis of expressive language dysfunction, although her inner language, communicative intent, attachment, and social motivation were intact (17).

Of the 17 autistic patients, 6 had been drug free for at least 1 month. Eleven autistic patients were treated with phenothiazines and/or butyrophenones. Of the 11 subjects in the control group, 10 were treated with identical drugs at similar dosage and 1 patient received diazepam.

Procedure

Cell-mediated response to human myelin basic protein was studied by the macrophage migration inhibition test.

Antigen. Human basic protein was extracted from the myelin of human brain according to Hirshfeld and associates (18). A concentration of 10 μg/ml of this preparation, which did not affect the normal migration of macrophages (18), was selected as the optimal dose of antigen in the macrophage migration test.

Lymphocytes. Twenty milliliters of heparinized venous blood were used. Lymphocytes were separated from whole blood by Ficoll-Isopaque density gradient centrifugation (19) and washed twice with phosphate-buffered saline and once with M-199 Hank's medium containing penicillin and streptomycin.

Macrophages. Macrophages were obtained from the peritoneal cavity of randomly bred guinea pigs that had been injected with 20 ml of paraffin oil 5 days earlier (20). The macrophages were washed twice with phosphate-buffered saline and once with M-199 Hank's medium and brought to a concentration of 8×10^7 cells/ml M-199 containing 15% heated fetal calf serum.

TABLE 1. Clinical Characteristics and Migration Indices of 17 Autistic Patients

Patient	Age (years)[a]	Sex	Drug Treatment (mg/day)	Migration Index[b]
1	12	M	Levomepromazine, 25	.71
2	11	M	Propericiazine, 8	.78
3	9	M	Levomepromazine, 25	.77
4	7	M	Propericiazine, 5	0
5	14	M	Propericiazine, 5; haloperidol, 5	.79
6	16	M	Thioridazine, 100	.73
7	16	F	Levomepromazine, 50	.89
8	16	F	Propericiazine, 5	.80
9	19	M	Propericiazine, 10	.51
10	18	M	Propericiazine, 10	.80
11	22	M	Thioridazine, 75	.73
12	12	M	None	.63
13	10	M	None	.70
14	9	M	None	1.0
15	9	M	None	.72
16	10	M	None	.72
17	17	M	None	.98

[a]Mean (±SD) age = 13.35±4.27.
[b]Mean (±SD) migration index = .78±.13.

Macrophage migration inhibition factor test. This test was performed by a modification of Rajapakse and Glynn's technique (21). A mixture of 8×10^6 packed lymphocytes with .5 ml of macrophage suspension (lymphocyte concentration in the suspension was 20%) was packed into capillary tubes by centrifugation and put into chambers filled with M-199 containing antigen and in chambers without the antigen. Each chamber contained one capillary tube. The migrations were allowed to proceed overnight at 37°C in an atmosphere of .5% CO_2. The results were obtained from the mean migrations of the duplicates in the different chambers, with a maximal divergency of 15% between them. The migration index was calculated according to the formula:

$$\text{Migration index} = \frac{\text{area of migration of lymphocytes + macrophages + antigen}}{\text{area of migration of lymphocytes + macrophages − antigen}} + \left[1 - \frac{\text{area of migration of macrophages + antigen}}{\text{area of migration of macrophages − antigen}}\right]$$

A migration index of .80 or less was considered as migration inhibition (22).

The migration index levels were evaluated statistically using Student's t test. This was a double-blind study where the migration inhibition factor results were not available to the clinicians; neither was the laboratory team apprised of the diagnoses until the end of the work.

RESULTS

Table 1 shows the clinical details and the migration indices of the autistic patients (group 1). Of the 17 autistic patients, 13 (76%) demonstrated inhibition of macrophage migration (migration inhibition factor from .51 to .80). The lymphocytes of the other 4 patients (patients 4, 6, 7, 17) did not affect the migration of macrophages. Six (35.3%) of the 17 patients were drug free for at least 1 month (patients 12 through 17). The mean (±SD) migration index of the drug-free autistic patients did not differ significantly from that of

TABLE 2. Clinical Characteristics and Migration Indices of 11 Control Subjects

Patient	Age (years)[a]	Sex	Diagnosis	Drug Treatment (mg/day)	Migration Index[b]
1	8	M	Mental retardation, deafness	Propericiazine, 5	.86
2	11	M	Schizophrenia, childhood type	Levomepromazine, 50	1.08
3	9	M	Emotional deprivation	Levomepromazine, 25	.93
4	9	F	Chronic organic brain syndrome	Levomepromazine, 25	1.04
5	6	M	Mental retardation	Propericiazine, 5	1.08
6	19	F	Mental retardation	Diazepam, 15	.88
7	17½	M	Schizophrenia, childhood type	Thioridazine, 100	.86
8	15	F	Aphasia, developmental type	Levomepromazine, 25	1.0
9	13	M	Schizophrenia, childhood type	Thioridazine, 100	.97
10	13	M	Schizophrenia, childhood type	Haloperidol, 10	1.05
11	11	F	Schizophrenia, childhood type	Thioridazine, 75	.90

[a]Mean (±SD) age = 11.95±4.02.
[b]Mean (±SD) migration index = .97±.09.

the patients under medication (.79±.16 versus .77±.12 migration index, respectively).

The clinical details and migration indices of the control group are summarized in table 2. All 11 patients had negative migration indices. A significant difference was obtained between the migration indices of the autistic patients and the control group (.78±.13 versus .97±.09) ($t=4.23$, $df=26$, $p<.001$).

The mean age of the autistic patients did not differ significantly from that of the control group (13.35± 4.27 versus 11.95±4.02 years).

DISCUSSION

The syndrome of autism is defined as a failure to develop normal social relationships, with communication disturbances of motor, social, adaptive, and cognitive developmental rates and sequences (2). We performed various investigations in order to elucidate possible biochemical etiologies of this disorder, including examination of metabolism of biogenic amines, amino acids, hormones, trace elements, plasma dopamine β-hydroxylase, and free fatty acid metabolism. No specific biochemical markers or deviations were demonstrated. Detailed reviews of biochemical studies in the syndrome of autism have been published in articles written by Cohen and Young (23), Ritvo (24), and DeMyer and associates (25).

Abnormal responses to sensory stimuli, such as auditory, vestibular, visual, tactile, olfactory, and proprioceptive, have been described. Whether these features are due to emotional disturbances (26, 27) or to organic brain lesion (6, 7) is still controversial. Neurological syndromes such as phenylketonuria, hypsarrhythmia, and encephalitis caused by fetal rubella infection that may result in autistic features, as well as other brain dysfunctions, raise the possibility that autism is associated with organic brain lesion (7). Extensive neurological examinations, including EEGs, demonstrated that signs of neurological dysfunction appear more frequently in autistic children than in normal children (28, 29).

In one study (30), computerized tomography (CT) scans revealed that 4 of 22 autistic patients had abnormal scans. This study and others (31, 32) suggested structural irregularities of the brain but could not indicate any specific, single abnormal pattern in this syndrome.

Damasio and Maurer (7) proposed the hypothesis that the features of autism (a combination of social, linguistic, cognitive, emotional, and behavioral disturbances) might be sequelae of dysfunction of bilateral neural structures, while others (3–6) suggested a lower involvement at the brainstem level.

In our present study, 13 of the 17 autistic patients tested showed a positive migration inhibition factor response to human basic protein, while none of the subjects in the control group responded to this antigen. This finding is, to the best of our knowledge, the first report of the existence of autoimmune response to brain antigen in autistic children. Many of the autistic children are exposed to antipsychotic drugs implicated in inducing autoimmune phenomena (33), and the assumption that such drugs might be the cause of an excess of human basic protein antigen could be made. Our remarks show that none of the 11 patients in the control group who were receiving similar medications and dosages responded to human basic protein. Furthermore, 6 of the 17 autistic patients had been drug free, and yet the percentage of patients who responded to human basic protein in this subgroup was the same as that for patients who were receiving drug treatment.

This result confirms that reported by one of us (34), suggesting that psychoactive drugs are not responsible for the appearance of a positive migration inhibition factor test to human basic protein in patients suffering from primary affective disorders and schizophrenia. The common phenomenon of sensitization to human basic protein both in the autistic syndrome and in long-term schizophrenia independent of drug treatment (19) suggests that an immunopathological reaction toward

brain tissue might be involved in the pathological process of these diseases.

Our finding of a positive migration inhibition factor test toward human basic protein in the group of autistic patients suggests that an undetectable brain lesion associated with autoimmunity may play a role in the pathogenesis of autism.

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