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Antibodies to Thymocytes in Sera of Patients with Schizophrenia

(organ-specific antigens/cytotoxicity test/immunofluorescence)

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ABSTRACT **Sera from healthy people and from patients with schizophrenia were heated at 56° for 30 min and then kept at 4°. These sera were then tested for cytotoxicity and by the indirect immunofluorescence method with thymocytes, bone-marrow cells, and lymph-node cells of adult C_3H mice. The cytotoxicity index for mouse thymocytes of sera from 60 healthy donors ranged from 0.00 to 0.4, with an average of 0.08. That of sera from 35 patients with schizophrenia ranged from 0.29 to 0.91, with an average of 0.71. The cytotoxicity index for mouse bone-marrow cells of sera from healthy donors ranged from 0.03 to 0.23, whereas that of sera from patients with schizophrenia ranged from 0.00 to 0.14. Sera of patients at dilutions of 1:8–1:32 displayed immunofluorescence of almost 100% of thymocytes and 58–78% of lymphocytes, whereas sera of healthy donors displayed none. We thus conclude that the sera of patients with schizophrenia contain antibodies against thymic antigens localized on thymocytes and thymus-derived lymphocytes.**

The occurrence of common organ-specific antigens in the brain and thymus is demonstrated by the fact that antisera prepared by immunization with brain tissue possess antithymocytic activity (1, 2). These antisera do not display species specificity; in particular, heterologous antisera prepared by injecting rabbits and goats with mouse, rat, or human brain tissue possess the activity not only with respect to thymocytes of the donor but of other species as well (2). This finding has been confirmed in experiments on absorption of antisera directed against brain (2). Concentration of brain-tissue antigens crossreactive with thymus antigens is rather high and even higher than their concentration in the thymus (3). At the same time, data are available indicating that apparently no complete immunological tolerance exists to the tissue-specific antigens of the thymus (4). This is indicated by the presence of thermolabile autoantibodies against thymus in numerous lines of mice (5) as well as by the appearance of autoantibodies to one's own thymocytes upon immunization with heterogeneous thymus, for example, some cases of autoantibody formation after immunization of AKR mice with C_3H mouse thymocytes (6).

Thus, it may be assumed that antibodies against thymocytes can be also produced in the pathological conditions accompanied by the autoimmunity to brain antigens, for instance, schizophrenia, autoimmune manifestations of which have been described by many authors (7, 8). The present work is an attempt to verify an assumption of the occurrence of antibodies against thymus in patients with schizophrenia.

Abbreviation: CI, cytotoxicity index; T lymphocytes, thymus-derived lymphocytes.

MATERIALS AND METHODS

The cytotoxicity test and the indirect immunofluorescence method were used with thymocytes, bone marrow, and lymph-node cells of adult C_3H mice. Sera from healthy donors and patients with schizophrenia were heated at 56° for 30 min and kept in the cold (4°).

For the cytotoxicity test, the cells derived from the thymus and bone marrow were suspended in saline with 5% isologous serum. Guinea-pig serum diluted 1:2 and absorbed with agar served as complement. Cell suspensions were filtered, packed by centrifugation, and diluted in saline up to 2 to 5 $\times$ 10^6 cells per ml. The reaction was performed according to the method of Gorer and O'Gorman (9) modified by Brondz (10). The standard condition under which the cytotoxicity reaction was run consisted of parallel performance of the test with sera of healthy donors and patients with schizophrenia on the same cell suspension and with the same lot of complement. In addition to native sera, the reaction was performed with the sera preliminarily absorbed with thymus and bone-marrow cells and with homogenates of the mouse brain and liver according to the method of Golub (2).

Results of the cytotoxicity test were calculated as follows:

$$\text{Cytotoxicity index} = \frac{A - B}{100 - B},$$

where A is the percentage of dead cells during incubation with serum and complement and B is the percentage of dead cells during incubation with serum and saline.

The indirect immunofluorescence reaction with mouse thymus and lymph-node cells was done according to the method of Weler and Coons (11) modified by Dorfman (12). Nonlabeled and fluorescein isothiocyanate-labeled eluate of rabbit antibodies against human gamma globulin was used in the reaction.

RESULTS

Table 1 presents the cytotoxicity indices (CI) for mouse thymocytes of sera of 60 healthy donors and 36 patients with schizophrenia. In all experiments the cytotoxicity of the complement itself did not exceed 5–7%. The CI of the sera of healthy donors fluctuated within a range of 0.00–0.4. It averaged 0.08. At the same time, CI of the sera of 35 patients with schizophrenia ranged from 0.29 to 0.91, averaging 0.71. The CI of the sera of one patient was 0.02 (see Table 1).

The cytotoxic activity decreases with serum dilution; sometimes it is retained at a dilution of 1:4 and 1:6. Absorption of the sera of patients with bone-marrow cells and liver

TABLE 1. *The cytotoxic activity of blood serum of patients with schizophrenia and healthy donors for mouse thymocytes*

No. of healthy donors	Cytotoxic index (CI)	No. of patients
8	0.00	0
6	0.01–0.02	1
7	0.03–0.04	0
9	0.05–0.07	0
12	0.08–0.10	0
3	0.11–0.15	0
11	0.16–0.28	0
2	0.29–0.31	2
2	0.32–0.42	2
0	0.43–0.53	6
0	0.54–0.59	4
0	0.60–0.64	5
0	0.65–0.68	5
0	0.70–0.84	7
0	0.85–0.91	4

Mean CI of sera of patients (M) = 0.71.
Mean CI of sera of healthy donors (M) = 0.08.

homogenate slightly inhibited the cytotoxic activity, whereas absorption with thymocytes and brain homogenate abolished their activity almost completely. Absorption of normal sera with thymocytes and brain homogenates did not alter the cytotoxic properties of the sera of healthy donors.

The CI of the sera from healthy donors with respect to the bone-marrow cells ranged from 0.03 to 0.23, whereas the sera of patients with schizophrenia ranged from 0.00 to 0.14.

Indirect fluorescence with the sera of healthy donors at a dilution of 1:8 and higher has shown that neither thymocytes nor lymph-node cells display any immunofluorescence (data not shown). At the same time the sera of patients at dilutions of 1:8, 1:16, and 1:32 give a bright fluorescence of almost 100% of thymocytes (Table 2).

The pattern of the reaction is a bright, continuous or dotted, ring-type membrane fluorescence. The reaction of immunofluorescence was inhibited when treatment of thymocytes with the sera of patients was followed by treatment with nonlabeled antibody against gamma globulin and, thereafter, with the labeled one. The reaction of immunofluorescence with lymph-node cells has revealed that among lymph-node cells from 65 to 78% of fluorescent cells were demonstrable when we used the diluted sera of patients with schizophrenia giving a 100% intensive fluorescence of thymocytes. The pattern and intensity of fluorescence of lymph-node cells differed from those of thymus cells.

DISCUSSION

The heated sera of healthy donors do not as a rule exert any marked cytotoxic effect on mouse thymocytes. At the same time, 35 of 36 tested sera of patients with schizophrenia were found to exert a clear-cut cytotoxic effect. The cytotoxic activity of the sera decreased with dilution but it was still retained at a dilution of 1:4–1:6. The cytotoxic activity of the same sera was considerably less for bone-marrow cells and did not exceed that of the sera of healthy donors.

The cytotoxic activity of the sera of patients with schizophrenia was abolished when the sera were absorbed with thymocytes and brain homogenates but not with bone-marrow cells and liver homogenates.

TABLE 2. *Comparative data on immunofluorescence of mouse thymocytes and lymph-node cells treated with different dilutions of the serum of a patient with schizophrenia*

Serum dilutions	Thymocytes (%)	Lymph-node cells (%)
1:8	100	78
1:16	100	65
1:32	98	58

Immunofluorescent staining has shown that the sera of patients whith schizophrenia possess immunoglobulin binding to the surface antigen of thymocytes. They also bind to surface antigens of 65–78% of lymph-node cells, giving less intensive and differently localized fluorescence. These data are in good agreement with the occurrence of T cells in mouse lymph nodes and localization of thymic antigens on the surface of thymus-derived lymphocytes. Viewed as a whole, these data indicate that, in fact, the sera of patients with schizophrenia seem to contain antibodies against thymic antigens localized on thymocytes and T lymphocytes, thus supporting the assumption we forwarded in the introduction.

The detection of antibodies possessing the cytotoxic activity for mouse thymocytes in the sera of patients with schizophrenia does not necessarily prove that these sera contain antibodies against human thymocytes; however, this assumption seems to be quite probable. The appearance of antibodies against thymus may be associated with immunization either with crossreactive brain antigens or with thymus antigens themselves. The presence of these antibodies can possibly affect T-lymphocytes, and a reduced level of blast transformation induced by phytohemagglutinin in leukocytes of patients with schizophrenia (13) may be a result of partial elimination (or inactivation) of T lymphocytes due to antibodies against thymus.

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