ANTITHYMUS ANTIBODIES IN THE BLOOD SERUM OF SCHIZOPHRENICS

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The blood sera of 36 schizophrenics and 60 mentally healthy donors were investigated by the cytotoxic test and the indirect immunofluorescence method. The blood serum of the schizophrenics was found to contain antibodies with cytotoxic activity against mouse thymocytes.

The presence of common organ-specific antigens in the brain and thymus is confirmed by the fact that sera obtained by immunization with brain tissue possess activity in vitro against thymocytes in the presence of complement [5]. Characteristically these sera do not exhibit complete species-specificity: heterologous sera obtained by immunizing rabbits or goats with brain tissue of mice, rats, and man possess activity against the thymocytes not only of the species whose brain was used for immunization, but also of other species. This is also confirmed by the results of experiments to study exhaustion of antibrain sera. The concentration of antigens cross-reacting with thymus in brain tissue is actually higher than their concentration in the thymus [8]. Meanwhile there is experimental evidence to show that complete immunologic tolerance to tissue-specific thymus antigens evidently does not exist. This is shown by the presence of thermolabile antithymus autoantibodies in many lines of mice [9] and also by the appearance of autoantibodies against the individual's own thymocytes after immunization with tissue from a foreign thymus as, for example, certain cases of autoantibody formation in AKR mice immunized with thymocytes from C3H mice [7].

The facts described above suggest that in pathological states accompanied by an autoimmune process aimed against brain antigens antibodies may perhaps also be produced against thymocytes. One such state is schizophrenia, autoimmune manifestations in which have been described by several workers [2].

The investigation described below was carried out to test this hypothesis that antithymus antibodies are present in schizophrenics.

EXPERIMENTAL METHOD

Blood was obtained from healthy donors and schizophrenics admitted to the clinical department of the Institute of Psychiatry, Academy of Medical Sciences of the USSR. Sera were prepared in the usual way, heated to 56°C for 30 min, and kept in the cold. Antithymus antibodies were determined by Gorer and O'Gorman's cytotoxic test [6] in the presence of complement in the modification of Brondz [1] and by Weller and Coons' indirect immunofluorescence method [10] in Dorfman's modification [4]. Thymocytes, bone marrow cells, and lymph gland cells from adult C3H mice were used to test the action of the serum.

The tests were carried out with native sera and also with sera previously adsorbed with thymus and bone marrow cells and with mouse brain and liver homogenates by Golub's method [5].

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TABLE 1. Cytotoxic Action of Blood Serum from Schizophrenics and Mentally Healthy Persons on Mouse Thymocytes (CTI)

CTI	Donors	Schizophrenics
0,00	8	0
0,01—0,02	6	1
0,03—0,04	7	0
0,05—0,07	9	0
0,08—0,10	12	0
0,11—0,15	3	0
0,16—0,28	11	0
0,29—0,31	2	2
0,32—0,42	2	2
0,43—0,53	0	6
0,54—0,59	0	4
0,60—0,64	0	5
0,65—0,68	0	5
0,70—0,84	0	7
0,85—0,91	0	4

Mean CTI (M): schizophrenics 0.71, healthy donors 0.08

EXPERIMENTAL RESULTS

The results of the cytotoxic test with the sera of 60 healthy donors and 36 schizophrenics are given in Table 1. The cytotoxic indices (CTI) of the action of sera from healthy donors and schizophrenics on thymocytes are given. The CTI were determined by the formula

$$\frac{A-B}{100-B},$$

where A represents the percentage of dead cells after incubation with serum and complement and B represents the percentage of dead cells after incubation with the serum and physiological saline. In no experiment did the cytotoxic action of the complement itself exceed 5-7%.

It will be clear from Table 1 that CTI for the sera of the healthy donors against mouse thymocytes varied from 0 to 0.42. Its mean value was 0.08. At the same time the CTI of the sera of 35 of the 36 schizophrenics studied varied from 0.29 to 0.91, with a mean value of 0.71. The CTI of the serum of one schizophrenic (Table 1) was 0.02. Against mouse bone marrow cells the CTI of the donors' sera varied from 0.03 to 0.23, but for the schizophrenics it varied from 0 to 0.10.

The cytotoxic action of the sera fell with dilution, sometimes persisting with dilutions of 1:4 and 1:6.

After adsorption of the schizophrenics' sera with bone marrow cells and liver homogenate their cytotoxic activity against thymocytes fell very slightly, whereas adsorption by thymocytes and brain homogenate removed practically all their activity. Adsorption of the healthy donors' sera by thymocytes and brain homogenate did not affect their cytotoxic activity.

The use of the immunofluorescence method showed that if the healthy donors' sera were used in dilutions of 1:8 or above, immunofluorescence neither of thymocytes nor of lymph gland cells could be observed. Meanwhile, if the schizophrenics' sera were used in dilutions of 1:8, 1:16, and 1:32, bright luminescence of almost 100% of the thymocytes was found. The luminescence appeared as a continuous or broken rim occupying the peripheral part of the cytoplasm. The immunofluorescence reaction was blocked if thymocytes treated with the schizophrenics' serum were covered with an unlabeled eluate of rabbit antiserum against human γ globulin, and a labeled eluate was then applied. The results of the immunofluorescence test with lymph gland cells were as follows: if diluted schizophrenics' sera were used, in which case luminescence of practically 100% of the thymocytes was observed, between 58 and 85% of the lymph gland cells were luminescent. The luminescence was weaker in intensity than in the thymocytes and its character was different.

These results show that heated healthy donors' sera as a rule have no marked cytotoxic action on mouse thymocytes. Meanwhile, a marked cytotoxic activity against thymocytes was observed in 35 of the 36 schizophrenics' sera tested.

The immunofluorescence experiments showed that the sera of schizophrenics contain immunoglobulins bound with the surface antigens of thymocytes. They are also bound with the surface antigens of 58-85% of lymph gland cells (depending on the dilution of the serum), although in that case the luminescence is less strong and is different in character. These results agree closely with the content of T-cells in the lymph glands of mice and the localization of thymocyte antigens on the surface of the T-lymphocytes.

The discovery of antibodies with cytotoxic activity against mouse thymocytes in the serum of schizophrenics is still not evidence that antibodies against human thymocytes are present. To study this problem direct experiments are necessary. However, it is known that if goats are immunized with human brain tissue, antibodies active against both human thymocytes and mouse thymocytes appear [5]. This is because the antigens of human brain include some which give a cross reaction with the antigens of thymocytes of man, mice, and other animals.

On the whole these results show that the serum of schizophrenics evidently contains antibodies against thymus antigens located on thymocytes and T-lymphocytes, confirming the hypothesis stated in the introduction. The appearance of these antibodies could be connected with immunization either with brain antigens cross-reacting with thymus antigens or with thymus antigens themselves. In any event the results suggest

that antithymus antibodies have a possible role in the immunological state of patients with schizophrenia. In particular, it can be postulated that the presence of these antibodies in the patients' sera could affect their T-lymphocytes. The existing evidence of the small proportion of peripheral blood lymphocytes of schizophrenics undergoing blast-transformation under the influence of cytohemagglutin [3] can be explained, in particular, by the partial elimination (or inactivation) of the T-lymphocytes of these patients by antithymus antibodies.

Finally, the results described above can probably be used in the laboratory diagnosis of patients with neurological and psychiatric diseases, especially schizophrenia. Preliminary results have shown that antibodies with the activity described above were not detectable in four patients with multiple sclerosis.

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