less frequently, class G, immunoglobulins possessing the properties of antibodies, but not damaging mature erythrocytes [1], was demonstrated on the surface of the peripheral erythrocytes of patients with PRCA. The role of these antibodies, directed against the Pr antigen of the erythrocyte membrane, in the pathogenesis of PRCA is not yet clear.

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REACTION OF SERA OF MYASTHENIA PATIENTS WITH THE SURFACE ANTIGENIC STRUCTURE OF THYMUS LYMPHOCYTES OF HEALTHY SUBJECTS AND PATIENTS WITH MYASTHENIA GRAVIS

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The blood of patients with myasthenia gravis contains antibodies against certain heteroorganic antigens of epithelial tissue of the human thymus. It has been shown by the immunofluorescence test, for instance, that the blood of patients with myasthenia contains antibodies against antigens of myoid cells common with antigens of skeletal muscle and myocardium [1, 5], and also against an antigen of the epithelial reticulum of the human thymus, common with the epithelium of the skin [2]. Deposition of immune complexes containing immunoglobulins of the M, A, and G classes has been found in sections of the thymus from patients with myasthenia by the direct immunofluorescence method [3]. Because of the distribution of the immune complexes it has been suggested that they contain antibodies against thymus tissue antigens and, primarily, against antigens of its lymphoid cells.

The object of the present investigation was thus to demonstrate antibodies against antigens of human thymus lymphoid cells in the spleen of patients with myasthenia and also to compare the reactions of these sera with lymphocytes from healthy human lymphoid organs and from the thymus of patients with myasthenia in order to discover any possible antigenic changes in the thymus lymphocytes in this disease.

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EXPERIMENTAL METHOD

The reaction of 112 sera from patients with myasthenia and 60 sera from healthy blood donors with sections through lymphoid organs (thymus, spleen, mesenteric lymph nodes) of persons dying from acute trauma at the age of 17-22 years, from lymphoid organs (thymus and spleen) of human fetuses (28-35 cm), and from the thymus of patients with myasthenia treated by thymectomy (18 cases) was studied by the indirect immunofluorescence method.

Tissue sections from the lymphoid organs 5-6 μ thick were cut in a cryostat (—20°C) from tissues of lymphoid organs frozen in petroleum benzin cooled to —70°C in a mixture of acetone and dry ice. Sections from all organs mounted on one slide, after drying for 30 min at room temperature, were treated with serum of a patient with myasthenia or a blood donor (dilution 1:20) for 18 h at 4°C, washed with buffered 0.85% NaCl solution (pH 7.0), and incubated for 30 min with an immunoglobulin fraction isolated from the serum of a donkey immunized with human immunoglobulins, labeled with fluorescein isothiocyanate (FITC). In some experiments sections of lymphoid organs treated with serum from a patient with myasthenia were incubated with FITC-labeled antibodies against human immunoglobulins, obtained by the method of Avrameas and Ternynck [4].

To determine the class of immunoglobulin to which the antibodies reacting with the antigenic structure of the human lymphocytes belonged, tissue sections of lymphoid organs, after treatment with the serum of a patient with myasthenia, were incubated with FITC-labeled immunoglobulin fractions isolated from the serum of rabbits immunized with human IgM, IgA, or IgG.

EXPERIMENTAL RESULTS

When a layer of serum from patients with myasthenia was poured on sections of healthy human thymus, spleen, and lymph nodes, fluorescence of punctate structures on the surface of the lymphoid cells of these organs was observed on sections of the thymus of patients with myasthenia (Fig. 1). A reaction with this antigenic structure of human lymphocytes was found in 42% of cases (47 of 112 sera); the antibody titer did not exceed 1:100, and in most cases it was 1:40. Sera of clinically healthy persons reacted with human lymphoid cells in 18% of cases (11 of 60 sera), but the antibody titer did not exceed 1:16.

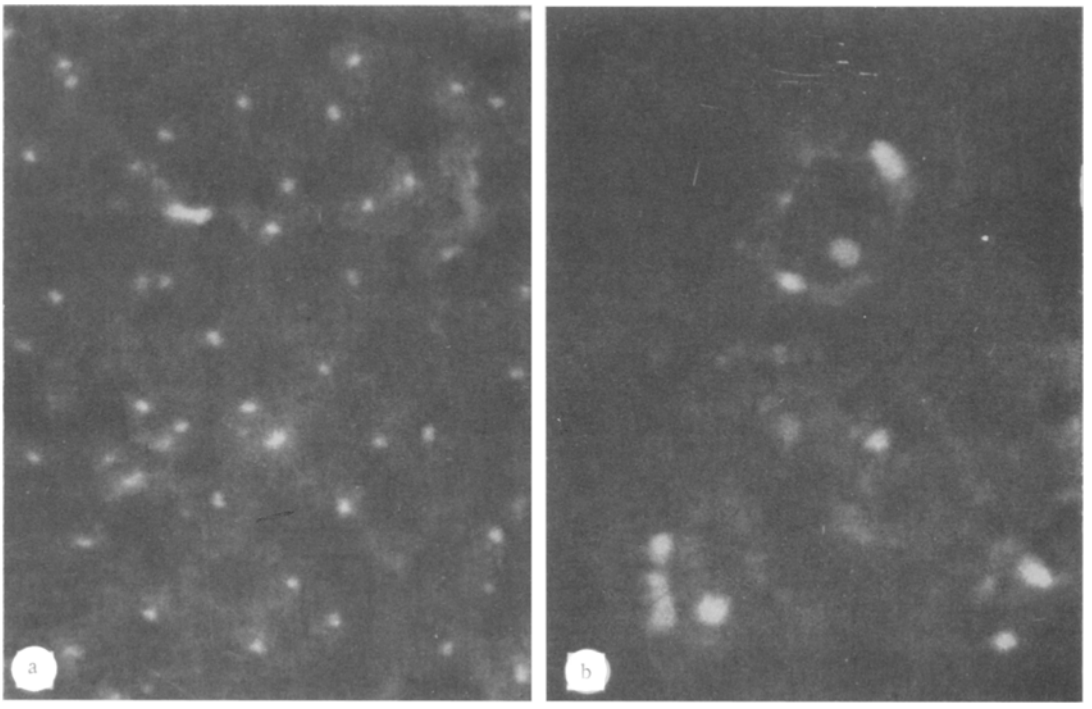

Fig. 1. Reaction of serum of myasthenia patient with punctate antigenic structure on surface of thymus lymphocytes of patients with this disease. Magnification: a) objective 40 (water immersion), ocular homal 3; b) objective 90 (oil immersion), ocular homal 3.

With labeled preparations against human IgM, IgA, and IgG it was found that antibodies reacting with the punctate antigenic structure of human lymphocytes belonged to the IgG class.

Although a punctate structure was detected on the surface of lymphocytes of all lymphoid organs from healthy donors studied, nevertheless, when the sections were treated with the same serum marked differences were found in the intensity of fluorescence of that structure on the surface of lymphocytes from different lymphoid organs. The sera reacted most strongly with the antigenic structure of splenic lymphocytes. The punctate structure on cells from lymph nodes gave somewhat weaker fluorescence than splenic lymphocytes, but no difference in the antigen content on lymphocytes of the peripheral lymphoid organs could be found by comparative titration. When the reactions of the sera of patients with myasthenia with the antigenic structure on healthy human thymocytes are characterized it should be noted that this reaction could not be detected when sera with low activity (1:20) were used and fluorescence was observed only after treatment of thymus sections with more active sera (1:40 or more). Under these circumstances the punctate formations were revealed on the surface of lymphocytes in the cortical and medullary zones of the lobules of the organ, but the intensity of fluorescence ofthis structure was much weaker than on lymphocytes of peripheral lymphoid organs.

The study of the reaction of the myasthenic patients' sera with sections of fetal lymphoid organsshowed thatthe punctate antigenic structure was well marked on the surface of lymphocytes of the fetal thymus and spleen, and in its intensity of fluorescence it was indistinguishable from the antigenic structure on cells of adult human peripheral lymphoid organs.

The immunomorphologic picture revealed in sections of the thymus of patients with myasthenia after incubation with the serum of a patient with this disease was very similar to that observed in sections of the healthy human thymus. Fluorescence of punctate antigenic formations, the number of which just as normally could vary from one to three, was observed under these circumstances on the surface of lymphocytes from the cortical and medullary zones of the lobules of the gland. However, when sections through the thymus of a myasthenia patient and a healthy donor were treated with serum from a patient with this disease, the antigenic structure of the patient's lymphocytes was characterized by a much greater intensity of fluorescence than that on healthy human thymocytes, and in this respect it was similar to the antigenic structure on lymphocytes of fetal lymphoid organs (thymus and spleen) and on lymphoid cells of healthy adult peripheral lymphoid organs (spleen and lymph nodes). Investigation of the reaction of myasthenic patients' serum with sections of the thymus of 18 patients with this disease showed that, despite the general rule according to which the antigenic structure on thymus lymphocytes of patients with myasthenia is characterized by a much greater intensity of fluorescence than healthy human thymocytes, the degree of expression of this structure in the patients is marked by considerable individual variations and correlate positively with the degree of development of the lymphoid tissue of the organ. Accordingly, the punctate antigenic structure on the thymocytes of patients with myasthenia, associated with lymphoid hyperplasia of the thymus, gave a much higher intensity of fluorescence than in cases of hypoplasia and atrophy of the lymphoid tissue of the gland.

The investigation thus showed that sera of patients with myasthenia react with a punctate antigenic structure on the surface of human lymphoid cells. The fact that fluorescence of this structure was observed in 42% of cases and that normal sera did not react under these circumstances is evidence that the reaction observed is due to the presence of antibodies against the antigenic structure of the lymphocytes in the sera of patients with myasthenia and is evidently not the result of nonspecific interaction of the Fc fragment of the serum immunoglobulins with the corresponding lymphocyte receptor.

According to the results described above, the antigenic structure of lymphocytes detectable with the aid of myasthenic patients' sera is formed early in embryogenesis, for it was clearly distinguishable on lymphocytes of the thymus and spleen of a human fetus 26-35 cm long. Comparative titration revealed no differences in the quantity of this antigen on spleen and thymus cells at this period of intrauterine development. No differences likewise were found in the quantity of this antigen on fetal lymphocytes and in adult human peripheral lymphoid organs (spleen and lymph nodes). In contrast to this, lymphocytes of the adult human thymus have a much lower content of this antigen than lymphocytes of adult human peripheral lymphoid organs and also than fetal lymphoid cells. These results are evidence that in the adult the quantity of this antigen on T cells increases only after their maturation and migration into peripheral lymphoid organs, whereas in the intrauterine period formation

of the punctate antigenic structure is completed at earlier stages of thymocyte differentiation.

The results of this investigation are evidence also that in myasthenia not only do antibodies appear against lymphoid cell antigen, but the content of this antigen on the patients' thymocytes increases compared with healthy human thymocytes. In this respect the thymocytes of patients with myasthenia resemble, on the one hand, lymphocytes of healthy human peripheral lymphoid organs and, on the other, lymphocytes of the fetal thymus and spleen. Further investigations are necessary to study the character of functional changes in the thymocytes during myasthenia — accelerated maturation or, conversely, a lower level of functional maturity, to which the observed antigenic modulation corresponds.

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