BOUND IMMUNOGLOBULINS OF CLASSES M, A, AND G STUDIED IN THE THYMUS OF PATIENTS WITH MYASTHENIA GRAVIS

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Deposits of granular material containing immunoglobulins (Ig) of the M, A, and G classes, were found by direct immunofluorescence in the thymus of patients with myasthenia. Treatment of sections of the thymus of myasthenic patients with unlabeled preparations against individual classes of human Ig inhibits the reaction of the granular material with homologous labeled preparations. Disappearance of fluorescence of the deposits also was observed in sections treated with glycine-HCl buffer, pH 2.8. These results suggest that the granular material consists of immune complexes in which IgM, IgA, and IgG act as antibodies and components of the thymus tissues as the antigen. The presence of bound Ig in the thymus is evidence that in myasthenia an autoimmune process directed against the tissues of this organ is involved.

KEY WORDS: thymus; myasthenia gravis; immunoglobulins; autoimmune diseases.

One of the morphological features of changes in the thymus in myasthenia is the formation of germinal centers – characteristic accumulations of lymphocytes around epithelial cells of the medullary zone of the lobules – in that organ. Some workers interpret the germinal centers as a manifestation of autoimmune thymitis, i.e., an immune process directed against the epithelial tissue of the thymus. In the opinion of these workers, the foci of lymphoid infiltration consist of "prohibited" clones of lymphocytes, immunologically competent relative to antigens of epithelial tissue which arise in the thymus under pathological conditions [6, 7]. According to another view, these collections of lymphocytes are regarded as a manifestation of inflammation of the epithelial tissue of the thymus and are analogous to inflammatory foci of infiltration formed in any injured organ. It is tentatively suggested that lymphocytes forming germinal centers penetrate into the thymus through blood vessels from peripheral lymphoid organs [9].

Evidence of involvement of the thymus in the pathological process in myasthenia is given by the presence of lymphocytes sensitized to antigens of the autologous thymus in the blood of these patients, together with antibodies against several hetero-organic antigens of the epithelial tissue of the organ [1-3, 8].

Besides morphological changes and the formation of autoantibodies and sensitized lymphocytes, one of the most important signs of the autoimmune process is the presence of immunoglobulins (Ig) bound with the antigens of the target organs.

Sections of thymus tissue from patients with myasthenia were studied by the direct immunofluorescence method in the present investigation in order to ascertain if they contained bound Ig.

EXPERIMENTAL METHOD

Fluorescence isothiocyanate-labeled pure antibodies against human IgG and immunoglobulin fractions isolated from the serum of rabbits immunized with human IgM, IgA, and IgG were used.* Pure antibodies were isolated from donkey serum against human IgG by the method of Avrameas and Ternynck [4]. Human Ig for the

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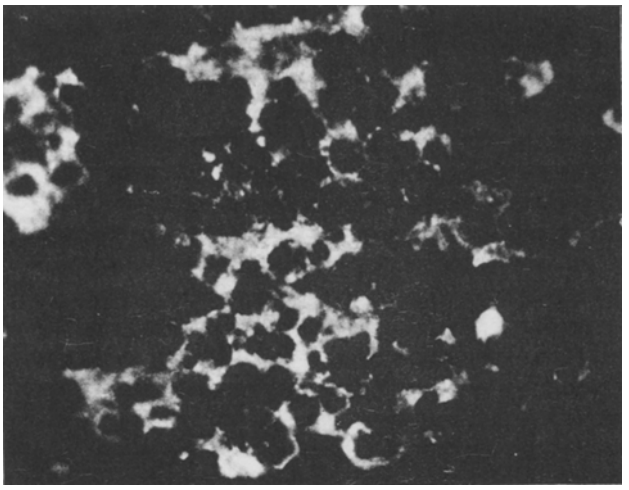

Fig. 1. Bound IgG in medullary zone of thymus of patient with myasthenia gravis. Here and in Fig. 2: direct immunofluorescence method, magnification: objective 30×, ocular 7×, homal 3×.

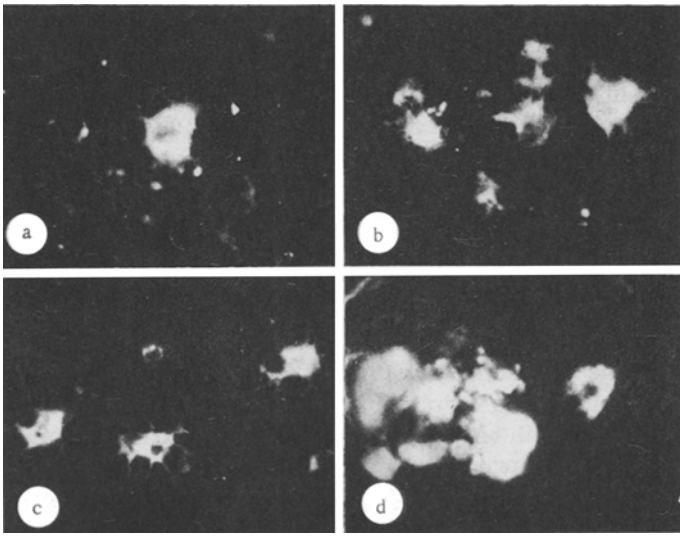

Fig. 2. Cells of plasma series synthesizing and secreting IgM in thymus of patient with myasthenia gravis: a, b, c) immune complexes containing IgM detected around plasma cells synthesizing this Ig and at borders of adjacent thymocytes; d) attachment of plasma cell synthesizing IgM to Hassall's corpuscle.

preparation of the immunosorbents (with the aid of glutaraldehyde) was obtained by Baumstark's method [5]. Sections through the thymus of patients thymectomized at the age of 10-35 years were studied by the direct immunofluorescence method. Sections through the thymus and spleen of persons dying from acute trauma at the age of 10-25 years were used as the control. The sections were cut to a thickness of 5-6 μ in a cryostat from unfixed tissue of organs frozen in petroleum ether at −70°C. Unfixed tissue sections of control organs and of the thymus of patients with myasthenia, mounted on the same side, were dried for 30 min at room temperature, treated for 45 min with the luminescent preparation, rinsed with buffered 0.85% NaCl solution (pH 7.0), and examined in the ML-2 luminescence microscope at a wavelength of 400 nm (SZS-7, BS-8, and FS-1 filters; ocular filters ZhS-18 and ZhZS-19; objective 40×, water immersion; ocular 15×). In some experiments before treatment with the labeled preparations, the sections were rinsed for 3-24 h with buffered 0.85% NaCl solution, pH 7.3, or with glycine-HCl buffer, pH 2.8.

The specificity of the reaction between the labeled preparations and the thymus sections from patients with myasthenia was verified by the inhibition test with appropriate unlabeled preparations. For this purpose the sections were treated for 30 min with the unlabeled preparations, rinsed for 10 min with buffered 0.85% NaCl solution, pH 7.0, and incubated for 45 min with labeled preparations against individual classes of Ig and with pure antibodies against human Ig.

EXPERIMENTAL RESULTS

On treatment of the unfixed sections of the thymus from patients with myasthenia with pure luminescent antibodies against human Ig and with immunoglobulin fractions against individual classes of Ig, extensive deposits of fluorescent material located in the intercellular spaces and at the borders of the lymphocytes were found in the medullary zone of the lobules of the organ (Fig. 1). The deposits of the different classes of Ig were dust-like, fine-grained, and granular in structure, and similar in their localization and distribution, as a result of which the morphological picture revealed in the medullary zone with the aid of each labeled preparation, including pure antibodies against human Ig, appeared identical. Deposits of Ig were discovered in all 22 organs tested, but their quantity varied both in each individual thymus and in the different lobules of the same thymus. Ig of the A and G classes in the thymus of patients with myasthenia were localized in the medullary zone, whereas granular material containing IgM was found as small foci and separate granules at the borders of the lymphocytes and also in the cortical zone of the lobules. Together with extracellular deposits of IgM, in the thymus of the patients with myasthenia there were cells whose cytoplasm contained this Ig (Fig. 2a-c). Intermediate forms of cells from small lymphocytes with diffuse fluorescence of their surface to plasmablasts and mature plasma cells with an extensive cytoplasm, filled with IgM, were observed. Lymphocytes and cells of blast type were found mainly in the lumen of the vessels in the medullary zone and in the perivascular region, whereas the more mature cells of the plasma series, synthesizing IgM, were localized in the parenchyma of the cortical and medullary zones of the thymus. Numerous granules containing IgM appeared around these cells, pointing to the active secretion of this Ig into the internal medium of the thymus, as a result of which new foci of IgM deposits were formed around the plasma cells (Fig. 2a-c). Plasma cells were more numerous in the medullary zone of the lobules than in the cortical zone and sometimes they were discovered against the background of extensive deposits of granular material containing IgM, and were attached to the Hassall's corpuscles (Fig. 2d).

When the content of IgM in the thymus was high, many cells with high secretory activity were found in it. If IgM was present in small amounts or absent, the cells synthesizing this Ig were few in number and most of them were lymphocytes or blast cells.

Preliminary treatment of the sections of the thymus from patients with myasthenia with unlabeled pure antibodies against human Ig and with immunoglobulin fractions against human Ig of classes M, A, and G largely inhibited the reaction of the sections with homologous labeled preparations but did not affect the intensity of the reaction of the sections with labeled preparations against Ig of other classes. Washing the sections of the thymus from myasthenia patients with buffered 0.85% NaCl solution (pH 7.3) for 24 h did not affect the character of their reaction with the labeled preparations. Treatment of thymus sections with glycine-HCl buffer, pH 2.8, for 24 h completely prevented fluorescence of the extracellular deposits of the granular material but did not affect the intensity of fluorescence of the cytoplasm of the cells synthesizing IgM.

The study of unfixed sections of the thymus and spleen from persons dying from acute trauma, by the direct immunofluorescence method showed no extracellular deposits of Ig in any of the 16 cases. Cells synthesizing IgM, IgA, and IgG were not found in the healthy human thymus. No such cells likewise could be found in unfixed sections of the spleen with the aid of anti-IgA- and anti-IgG-preparations or with anti-Ig-antibodies. By contrast, when unfixed sections of the spleen were treated with the anti-IgM-preparation, numerous cells of the plasma series containing this Ig in their cytoplasm were discovered in them. However, no degranulation of the cells or extracellular deposits of IgM were found in the spleen.

Deposits of granular material in the medullary zone of the lobules of the organ were thus found by the direct immunofluorescence method using luminescent antibodies against human Ig in unfixed sections through the thymus of patients with myasthenia in all 22 cases studied. The use of luminescent preparations against individual classes of human Ig showed that the granules contained Ig of classes M, A, and G. At the same time, cells of the plasma series synthesizing and secreting IgM were found in the thymus of the patients with myasthenia. Plasma cells synthesizing this Ig also were detected in the normal spleen. However, no granules containing IgM were found around them. This indicates that IgM secreted by plasma cells in the thymus of patients with myasthenia and in the normal spleen is in different states. In the thymus of patients with myasthenia the

granular material evidently consists of immune complexes, in which components of the thymus tissues behave as the antigen and IgM as the antibody. Besides the morphological features distinguishing the reaction of labeled preparations with sections of the thymus from patients with myasthenia, other evidence in support of this hypothesis is given by the fact that the granular material containing IgM is destroyed by the action of an acid buffer on the section. The impression is created that the precursors of cells synthesizing IgM are formed not in the lymphoid tissue of the thymus, but in the peripheral lymphoid organs, and that they penetrate into the thymus through the blood vessels. This is evidently the reason why small lymphocytes and cells of blast type are detected in the lumen of the vessels and in the perivascular region, whereas the more mature cells of the plasma series are found in the parenchyma of the thymus. The possibility likewise cannot be ruled out that autoantibodies synthesized in the peripheral lymphoid organs may also penetrate from the blood stream.

Although cells synthesizing these Ig could not be found by means of the anti-IgA- and anti-IgG-preparations used in the thymus of the patients, just as in the control organs (healthy human thymus and spleen), nevertheless the mechanism of formation of deposits of all three classes of Ig is evidently the same. This conclusion is supported by observations showing that immune complexes containing Ig of all three classes are destroyed by the action of an acid buffer on the sections, and also by observations of other workers who showed that the thymus of patients with myasthenia contains plasma cells synthesizing IgM, IgA, and IgG [7, 11].

The final point to be made is that the predominant location of the immune complexes to correspond with the boundaries of the thymocytes indicates that the antibodies are directed against the surface antigen of these cells. However, this location of the complexes, as well as their deposition in the internal medium of the thymus without any connection with lymphocytes, can be explained on the grounds that the antigen belongs to the epithelial cells or is a product of their secretion. Despite the fact that the presence of bound Ig in the thymus is evidence in support of the existence of autoimmune thymitis in myasthenia, the existing data thus do not allow any conclusion to be drawn regarding the tissue source of the antigen against which this process is directed.

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