BOUND IMMUNOGLOBULINS AND IMMUNE COMPLEXES IN THE THYMUS OF MYOPATHY PATIENTS

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KEY WORDS: immunopathology; thymus; immune complexes; myopathy.

In certain diseases damage to one organ is accompanied by the appearance of bound immunoglobulin in the injured cells and by deposition of granules of immune complexes. Previously the writers showed by the direct immunofluorescence method that deposition of immune complexes containing IgM, IgA, IgG, and complement in patients with rheumatic fever and myasthenia takes place in the thymus, the central organ of the lymphoid system [2, 3]. In the thymus of patients with rheumatic fever immune complexes are deposited in the cortex, i.e., around the undifferentiated lymphocytes of the organ, whereas in myasthenia they are found only in the medulla, i.e., in the zone where the functionally most mature lymphocytes of the thymus are located. In recent years, by analogy with myasthenia, attemps have been made to treat another neuromuscular disease, namely progressive muscular dystrophy (myopathy), by thymectomy.

This paper describes the results of a study of the thymus of myopathy patients by the immunofluorescence method in order to detect fixed immunoglobulins and immune complexes in the tissue structures and internal medium of the gland.

EXPERIMENTAL METHOD

Sections through the thymus of patients with Duchenne's myopathy (18 cases) and patients with Erb's myopathy (six cases), on whom thymectomy was performed at the age of 5-22 years, were studied by the direct immunofluorescence method. Sections of the thymus from children undergoing operations for congenital heart defects at the age of 4-15 years (12 cases) and of the thymus from persons dying from acute trauma at the age of 8-22 years (22 cases) were used as the control. To detect bound immunoglobulins and immune complexes, monospecific sera against immunoglobulins of the M, A, and G classes, labeled with fluorescein isothiocyanate (FITC), obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, were used. The presence of complement was determined with the aid of the FITC-labeled globulin fraction isolated from the serum of a goat immunized with C3 component of complement (from Hyland, USA). To remove any possible contamination with heterophilic antibodies against thymus tissue antigens all the labeled preparations were first adsorbed with human thymus tissue homogenate for 2 h at room temperature.

Sections of the thymus of a myopathy patient and of a control organ, mounted on the same slide, were dried for 1 h at room temperature, washed for 20 min in a stream of buffered physiological saline (BPS), pH 7.4, and treated with the labeled preparations for 18 h at 4°C. At the end of incubation the fractions were washed for 20 min in BPS and mounted in buffered glycerol, pH 7.4.

Hassall's corpuscles in the thymus of the myopathy patients were revealed by the indirect immunofluorescence method using blood serum from myasthenia patients and healthy blood donors, for such sera have been shown to react with antigens common to thymic corpuscles and the epidermis [1]. Sections of the thymus from myopathy patients and the control group were treated for 18 h with serum from a myasthenia patient or healthy subject (dilution 1:20),

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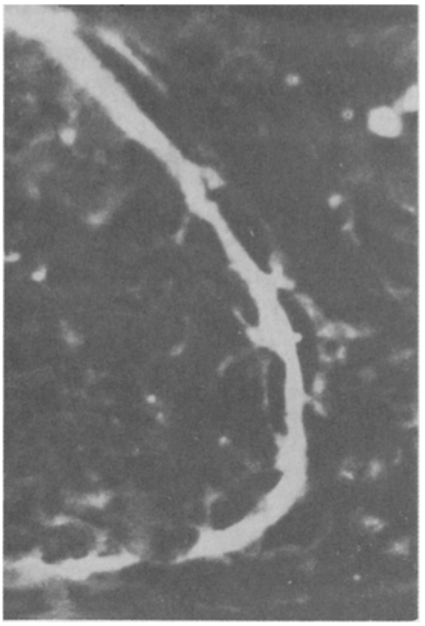

Fig. 1

Fig. 2

Fig. 1. Fixation of IgG in basement membranes surrounding thymus lobule.

Fig. 2. Bound IgG in cytoplasm of cambial cells of epithelial tissue in cortex of thymus lobule.

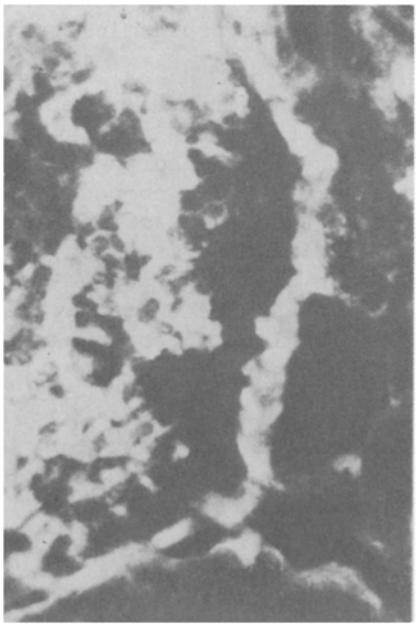

Fig. 3

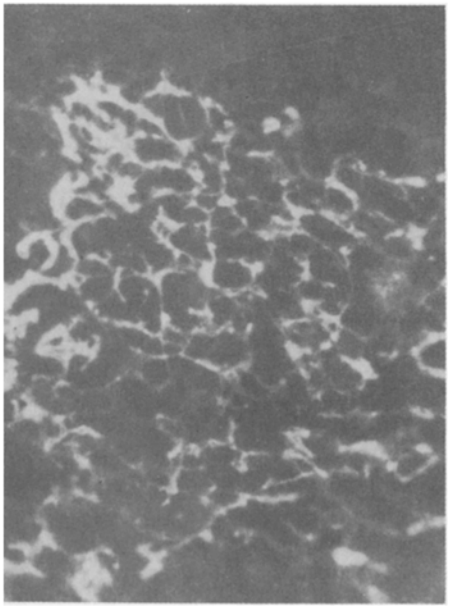

Fig. 4

Fig. 3. C3 component of complement in basement membrane and in cytoplasm of adjacent cells.

Fig. 4. Immune complexes containing IgM in intercellular spaces along boundaries of lymphocytes in corticomedullary zone of thymus of a myopathy patient.

containing antibodies against antigens of the epithelial tissue of the thymus, and after washing they were incubated for 45 min with FITC-labeled antibodies against human IgG.

EXPERIMENTAL RESULTS

Fluorescence of membrane-like structures (evidently basement membranes) located between the cambial layer of the cortical epithelial cells and the connective-tissue capsule surrounding the lobule (Figs. 1-4), was observed in sections of the thymus from myopathy patients, treated with labeled preparations against IgM, IgA, IgG, and the C3 component of complement. Bound immunoglobulins also were present in membrane-like formations located on the boundary between the cortex and medulla. There they were more twisted in shape and penetrated deeply in the form of looped structures into the medullary layer. Additionally fixed immunoglobulins and complement were seen in the cytoplasm of epithelial cells adjacent to the basement membranes, but they could also be cubical or oval in shape, with a base resembling a pedicle, by means of which they were attached to the basement membranes (Fig. 2). In many cases fixation of immunoglobulins and complement on membranes was accompanied by detachment of the epithelial cells adjacent to them (Fig. 3). Bound IgG was found in membrane-like structures in 80% of cases. In 50% of cases fixation if IgG also was observed in the cytoplasm of epithelial cells adjacent to them. In 45% of cases IgA, and in 30% of cases the C3-component of complement also were found in these structures. Bound IgM was not found in the basement membranes or in the epithelium.

Besides these observations, focal deposits of immune complexes containing immunoglobulins of the M, A, and G classes and the C3 component of complement also were found in the thymus of myopathy patients in 30% of cases. Material containing immunoglublins and complement in the form of fine granules (1-2 μ) and also of larger formations (5 μ) filled the intracellular spaces around the individual groups of lymphoid cells in the corticomedullary zone of the lobules of the gland (Fig. 4). In outer layers of the cortex and also right in the center of the medullary layer of the lobule no deposits of immune complexes were found. Numerous cells of the plasmacyte series were found in the interlobular connective tissue and in the corticomedullary zone of the parenchyma of the thymus in myopathy patients. IgM, IgA, and IgG were discovered in the form of granules in the cytoplasm of these cells. Oval cells morphologically similar to mast cells also were found here. The cytoplasmic granules of these cells contained the C3 component of complement.

Treating sections of the thymus with blood serum from myasthenia patients or healthy subjects, containing antibodies against antigens common to thymic epithelium and the epidermis, showed elective staining of Hassall's corpuscles. The thymic corpuscles in sections of patients with myopathy consisted mainly of large cysts (200-500 μ), composed of chambers filled with keratinized material and surrounded by one or two layers of very elongated cells. Fusion of small Hassall's corpuscles often was observed, with the formation of larger cysts, and sometimes the contents of the chambers escaped through gaps between the cells into the internal medium of the gland. Cyst-like Hassall's corpuscles were extremely rare in sections of the thymus in the control group, and freely lying keratinized material was even less common, for it was present only in small amounts and chiefly in the thymus of subjects over 20 years of age.

The results of these investigations thus demonstrate profound changes in the epithelial and lymphoid tissue cells of the thymus in progressive muscular dystrophy. One of the most characteristic immunomorphological manifestations of damage to the epithelial tissue of the thymus in this disease is fixation of immunoglobulins and complement in the basement membranes and in the cytoplasm of epithelial cells adjacent to them. The discovery of immunoglobulins and complement in the subcapsular (i.e., cambial) epithelial cells is evidence that the pathological process in myopathy involves epithelial cells in the earliest stages of differentiation. Another important feature of the changes in the epithelial tissue of the thymus in this disease is the well-marked process of cystic degeneration of Hassall's corpuscles, evidently reflecting the more rapid differentiation and, consequently, keratinization of the epithelium of the gland. The epithelial tissue of the thymus is known to play a decisive role in functional maturation of its lymphocytes. Hence it follows that injury to epithelial tissue may disturb differentiation of thymic lymphocytes and, consequently, may disturb their function. The fact that cells of the lymphoid tissue of the thymus are involved in the pathological process in myopathy is demonstrated by focal deposition of immune complexes in the corticomedullary zone of the thymic lobules. The distribution of immune complexes in the intercellular spaces around lymphoid cells suggests that antibodies against the antigens of these cells are present in the complexes.

Besides myasethenia and rheumatic fever, myopathy is thus another disease in which immune complexes are deposited in the thymus. The location of the complexes in different zones of the thymic lobule is evidence that different subpopulations of thymic lymphocytes undergo changes in these diseases. In conclusion it must be pointed out that, although the causes of the changes in thymic tissues in myopathy are not yet known, the very fact that such changes are present suggests that immunopathological disturbances due to injury to the central organ of the lymphoid system play an important role in the development and course of this disease.

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EFFECT OF MOLECULAR WEIGHT OF AGGREGATED IMMUNOGLOBULINS ON THEIR COMPLEMENT-FIXING ABILITY

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Reliable evidence of the pathogenetic role of immune complexes (IC) in several infectious, immune, and autoimmune diseases has recently been obtained [4, 7, 9]. Despite much research into the role of IC in the pathogenesis of these diseases, the problem of which physicochemical properties determine the pathogenetic properties of the IC, and of what determines their clearance, still remains unexplained.

It was accordingly decided to study the effect of size of IC on their ability to fix complement, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

As models of IC of different molecular weight (M) aggregated human IgG, obtained from normal human blood serum by ion-exchange chromatography on DEAE-cellulose was used.

Aggregation of the IgG was carried out at 63°C for 20 min. Since M of the aggregates arises during aggregation with an increase in the protein concentration [3, 8], to obtain aggregates of different M original solutions of IgG with different initial concentrations, from 0.5 to 2 mg/ml, were used. M of the aggregates was determined nephelometrically [2]. As a result, solutions with mean weights of aggregates amounting to 7, 10, and 15 times the M of IgG were obtained.

A freshly prepared solution of standard lyophilized guinea pig serum, from the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, was used as complement.

The complement-fixing activity of IC of different M was determined by thermistography [1]. The intensity of the reaction was assessed by the value of tan α, where α is the angle of slope of the experimental curve relative to the control curve. The complement-fixation reaction of IC with activated complement and the reaction between unaggregated IgG in initial concentrations in the solution and native complement served as the control.

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