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IMMUNE COMPLEXES IN THE THYMUS IN RHEUMATIC FEVER

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Germinal centers — focal concentrations of lymphoid cells in the corticomedullary and medullary zones of the lobules, are found in the thymus in certain diseases. These formations are particularly frequent in the thymus of patients with myasthenia, but they are also found in systemic lupus erythematosus (SLE), rheumatic fever, and thyroiditis [2, 3, 5-9]. According to some investigators, these concentrations of lymphoid cells consist of "prohibited clones" of lymphocytes which arise in the thymus under pathological conditions and are immunologically competent with respect to the antigens of this gland [2, 5]. According to another point of view, concentrations of lymphoid cells in the parenchyma of the thymus are analogous to lymphoid infiltrations which may be formed in any injured organ as a result of penetration of lymphoid cells from the blood stream [5]. Injury to the tissue of an organ is known to be accompanied by the appearance of bound immunoglobulins in the damaged cells and by the deposition of immune complexes in the internal medium of the organ. Deposition of immune complexes containing IgM, IgA, IgG, and complement have been found in the medullary zone of the thymus in patients with myasthenia [1]. Immune complexes also have been found in the thymus tissues of patients with SLE [4].

In the investigation described below a search was made for immune complexes in the thymus tissues of patients with rheumatic fever.

EXPERIMENTAL METHOD

Immune complexes were detected by the use of fluorescein isothiocyanate-labeled globulin fractions isolated from the blood serum of animals (rabbit, sheep) immunized with myeloma IgM and IgA or normal human IgG (from the N. F. Gamaleya Institute of Epidemiology and Microbiology). The presence of complement in thymus sections of rheumatic fever patients was determined by means of a fluorescein isothiocyanate-labeled globulin fraction isolated from the serum of a sheep immunized with the C3 component of human complement (from Hyland, USA). Thymus sections from rheumatic fever patients undergoing operations for valve implantation at the age of 8-18 years (27 cases) and from persons dying from acute trauma at the age of 8-22 years (16 cases) were studied. Frozen sections 5-6 μ thick were prepared from unfixed thymus tissue frozen in petroleum ether at −86°C. Unfixed sections from the thymus of a rheumatic fever patient and from a control thymus, placed on the same slide, were washed for 30 min in a current of buffered physiological saline (BPS), pH 7.5, and treated with the labeled preparations for

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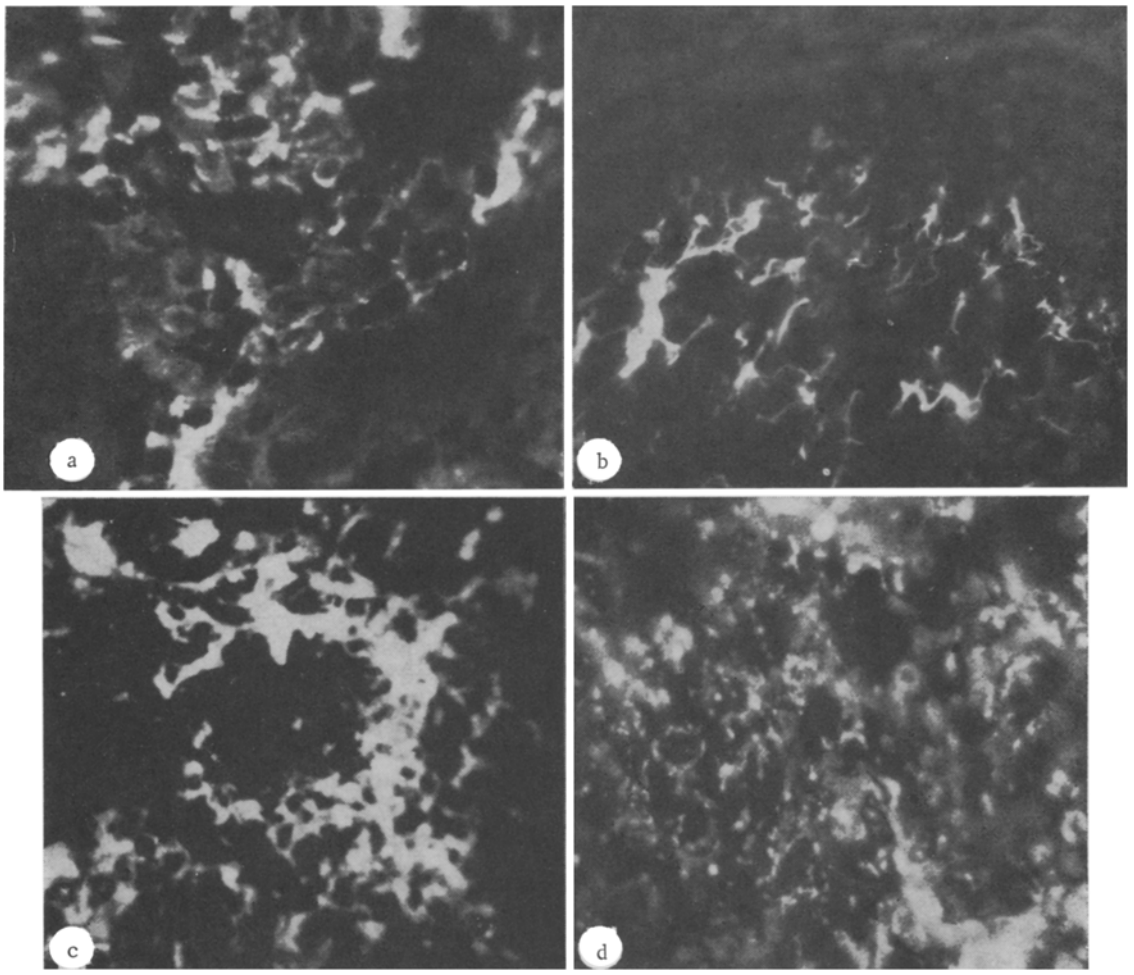

Fig. 1. Immune complexes in thymus of rheumatic fever patients: a) demonstration of granules containing IgM around least differentiated lymphoid cells forming outer layers of cortical zone; b) immune complexes containing IgA with the appearance of vermiform convoluted formations visible in intercellular spaces between lymphocytes of deeper layers of cortical zone; c) conglomerates of immune complexes containing IgG in internal medium and around lymphocytes of corticomedullary zone; d) section through thymus from same patient (c), fluorescence of granules containing C3 component of complement, located on surface and around lymphocytes of corticomedullary zone of thymus. Direct immunofluorescence method. Water immersion. Magnification: objective 40, ocular homal 3.

18 h at 4°C. At the end of incubation the sections were washed for 20 min with running BPS and mounted in 60% glycerol, pH 7.2. In control experiments thymus sections from rheumatic fever patients were first treated with glycine-HCl buffer, pH 2.8, or with BPS, pH 7.5, for 18 h at 4°C, then incubated with the labeled preparations.

EXPERIMENTAL RESULTS

When unfixed sections of the thymus from rheumatic fever patients were treated with labeled populations against individual classes of immunoglobulins and against the C3 component of complement, bright fluorescence of focal deposits of immune complexes was found in the cortical and medullary zones of the lobules of the gland; these complexes were located in the intercellular spaces and at the boundaries of the lymphoid cells (Fig. 1). Material containing immunoglobulins and complement was detected in the form of very small dust-like formations, granules measuring 1-2 μ (Fig. 1a-c) or larger conglomerates, attaining a size of 5-6 μ and with a curious vermiform and clumped appearance (Fig. 1d). Immune complexes were found around the least differentiated lymphoid cells, located beneath the capsule of the lobule and forming several of the first layers of lymphoid cells of the cortical zone

(Fig. 1a, b), in the intercellular spaces, and on the boundaries of the lymphocytes forming the deeper layers of the cortical zone (Fig. 1b), and also in the corticomedullary zone, where they surrounded lymphocytes of the lower layers of the cortical zone and the outer layers of the medullary zone (Fig. 1c, d). The dimensions of the focal deposits of immune complexes varied considerably and in most cases they were quite large, extending over large groups of lymphoid cells.

Because of the morphological similarity and identical distribution of the immune complexes in the internal medium of the thymus the immunomorphological picture revealed by the use of labeled preparations against each class of immunoglobulin and complement was identical. Most frequently immune complexes containing IgG (63% of cases) were found in the thymus of rheumatic fever patients, IgA was found rather less frequently in their composition (47%), and in 24% of cases the immune complexes contained IgM. The C3 component of complement was found in four of ten immune complexes, and both IgG and (or) IgA could accompany it in the composition of the granules. Focal deposits of granules containing complement were found much less frequently and were much smaller in size than the deposits of material containing IgG or IgA discovered in sections of this same thymus.

Besides deposition of immune complexes in the cortical and medullary zones of the lobules of the thymus in rheumatic fever patients, numerous plasma cells synthesizing IgM, IgA, or IgG also were found. Concentrations of plasma cells also were observed in the interlobular connective tissue, from which they penetrated into the parenchyma of the organ. Sometimes cells of the plasmacyte series could also be observed in the lumen and wall of the blood vessels in the thymus of rheumatic fever patients. With the aid of the luminescent preparation against the C3 component of complement, brightly fluorescent oval cells with finely granular cytoplasm could be seen in the interlobular connective tissue and in the parenchyma of the medullary zone of the thymus of rheumatic fever patients.

In the control experiments, partial loss of fluorescence of the granules of immune complexes was observed in sections of the thymus of rheumatic fever patients triggered beforehand with glycine-HCl buffer, pH 2.8. Washing the sections with BPS, pH 7.5, had no effect on the brightness of fluorescence of the immune complexes. In sections through the thymus of persons dying from acute trauma, numerous lymphoblasts with IgG in their cytoplasm were detected on an area of interlobular connective tissue. In two cases single plasma cells producing IgA or IgG were found in the parenchyma of the thymus. Plasma cells synthesizing IgM were not found in the healthy human thymus. No reactions were found between the anti-C3 preparation and sections of the control thymus.

The results of a study of the thymus of rheumatic fever patients by the immunofluorescence method thus indicate that in this disease a deep-seated pathological process takes place in the gland, one manifestation of which is the formation and accumulation of a considerable quantity of immune complexes in the internal medium of the thymus. Besides deposition of immune complexes in the thymus of rheumatic fever patients, many cells of the plasmacyte series synthesizing IgM, IgA, and IgG also were found. The particular locations of these cells indicate that they penetrate into the thymus via the blood vessels, and on reaching it they secrete antibodies which are evidently directed against antigens of thymus tissues. It is also possible that some antibodies are synthesized by the cells of peripheral lymphoid organs and that these penetrate into the thymus from the blood stream. The location of the immune complexes in the intercellular spaces among the boundaries of the lymphocytes of the cortical and cortico-medullary zones suggests that the antibodies in their composition are directed against surface antigens of thymocytes or the factors which they secrete into the internal medium of the thymus.

In previous investigations the writers showed the presence of extensive deposits of immune complexes and large numbers of plasma cells in the thymus of patients with myasthenia gravis [1]. However, in that disease immune complexes are located in the center of the medullary zone of the lobules along the boundaries of the medullary lymphocytes, functionally the most mature lymphocytes in the thymus, whereas in rheumatic fever immune complexes are found in the cortical and cortico-medullary zones, i.e., around the undifferentiated lymphoid cells of the thymus. These differences in the location of the immune complexes in the thymus of patients with rheumatic fever and myasthenia are evidence that the function of the lymphocyte subpopulations of the thymus be disturbed in these diseases.

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