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## ANTIBODIES AGAINST COMMON ANTIGEN OF EPITHELIAL TISSUE OF HUMAN THYMUS AND SKIN EPIDERMIS IN MYASTHENIA GRAVIS

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Experiments by the indirect immunofluorescence method showed that the sera of patients with myasthenia gravis in a high percentage of cases react with cells of epithelial type of the human thymus. By absorption of the sera with suspensions of epidermal cells and tissue homogenates of several human organs it was shown that the antigen of the epithelial cells with which the sera of patients with myasthenia react belongs to the epidermal heteroorganic antigens of the thymus, i.e., it is common to the epithelium of the thymus and the epidermis of the human skin. The presence of antibodies against epithelial tissue cells of the thymus in the blood serum of patients with myasthenia gravis suggests that in this disease an immunopathological process takes place, aimed against thymus tissue antigens, including against the heteroorganic structures of its epithelium.

KEY WORDS: myasthenia gravis; epithelium of the thymus; epidermis of the skin; cross-reacting antigens and antibodies.

The serum of patients with myasthenia gravis is known to contain antibodies against antigens of the myoid cells of the thymus common with antigens of skeletal muscle and myocardium [1, 2, 6]. In this disease, as in other autoimmune processes (rheumatic fever, ulcerative colitis, multiple sclerosis), the serum reacts in a

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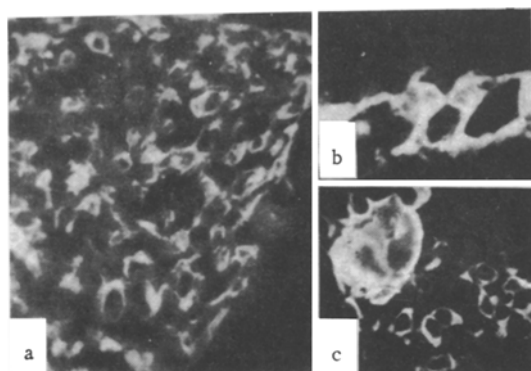


Fig. 1. Section through human thymus tissue treated with serum from patient with myasthenia gravis: a) fluorescence of cytoplasm of cells of epithelial reticulum of thymus giving off processes; b) fluorescence of cytoplasm of cells of basal layer in cortical zone of lobe, base of which is shaped like a pedicle; c) attachment of cells of epithelial reticulum to Hassall's corpuscles; 40 $\times$ .

higher percentage of cases than normal with a common antigen shared by the cells of Hassall's corpuscles and the keratin of the epidermis of the skin [3]. It has also been shown that in these autoimmune diseases, including in myasthenia gravis, the patients' blood contains antibodies against cells of the epithelial reticulum of the human thymus [4].

In this investigation the tissue and organ nature of the epithelial antigen of the human thymus with which the sera of patients with myasthenia gravis react was studied.

#### EXPERIMENTAL METHOD

Experiments were carried out by the indirect immunofluorescence method using pure antibodies against human immunoglobulins labeled with fluorescein isothiocyanate (FITC) [5].

The reaction of 90 sera of patients with myasthenia and 50 sera of clinically healthy subjects (blood donors) with tissue sections from the thymus and skin of people dying from injury at the age of 10–25 years (12 cases), the thymus of infants dying from infectious diseases at the ages of 9 and 18 months (two cases), and the thymus of 14–20-week-old human embryos was studied. Rabbit, guinea pig, bovine, C3H mouse, and chicken thymus glands also were used. Sections of thymus tissue were treated with serum (diluted 1:10) for 18 h at 4°C, washed for 30 min with buffered 0.85% NaCl solution, pH 7.0, and incubated for 30 min with labeled antibodies against human immunoglobulins. To study the organ specificity of the epithelial tissue antigen of the thymus the sera were absorbed with tissue homogenates of spleen, brain, heart, liver, kidney, and thymus, a suspension of group AB erythrocytes, and a suspension of keratinizing epidermis and human fetal epidermis. Keratinizing epidermis was obtained by scarification of the skin surface of the healthy donors. Human fetal epidermis was obtained by a modified method of Medawar [7]. Pieces of skin were treated with 1% trypsin solution for 1–1.5 h at 37°C. The epidermis, separated from the dermis, was transferred by a scalpel into buffered 0.85% NaCl solution, pH 7.0, containing 0.05% EDTA in the final concentration and, after repeated washing, was used in the absorption experiments. The patient's blood serum and tissue homogenate were taken in the ratio of 2:1. The mixture was incubated for 1 h at 37°C and allowed to stand overnight at 4°C. In the absorption experiments mainly those sera which reacted with myoid cells as well as with cells of the epithelial reticulum were used. Under these conditions preservation of the reaction with myoid cells was used as an additional control of the specificity of absorption of the sera by the epidermal cell suspension.

To identify the class of immunoglobulin to which the antibodies reacting with the epithelium of the human thymus belonged, sections of thymus treated with patient's serum were incubated with FITC-labeled fractions of immunoglobulins of rabbits immunized with human IgA, IgM, and IgG. These preparations were generously provided by Professor Chorszelski from the Dermatological Institute of the Polish Academy of Sciences.

## EXPERIMENTAL RESULTS

When sera from patients with myasthenia were layered above sections of the human thymus intense fluorescence of the cytoplasm of the epithelial cells was observed in the cortical and medullary zones of the lobes of the organ (Fig. 1a, b, and c). The distinguishing morphological feature of these cells was that they possess many processes, by means of which they made contact with each other to form the characteristic pattern of an epithelial reticulum (Fig. 1a). One of the processes could be shaped like a pedicle, with the aid of which the cell rested on the inner surface of the capsule of the lobe, on the connective tissue septa growing into the medullary zone, and on the adventitial membrane of blood vessels (Fig. 1b). In some cases a basement membrane appeared between the base of the pedicle and the underlying connective tissue. Epithelial cells with which the sera of the patients with myasthenia reacted in the medullary zone of the lobes of the thymus were attached in large numbers to the Hassall's corpuscles (Fig. 1c).

Only the most active sera reacted with the epithelial cells of the human embryonic thymus and the intensity of the reaction was much weaker than with sections of the infantile and adult human thymus. An even weaker reaction was observed with chick thymus epithelium. With sections of rabbits, bovine, guinea pig, and mouse thymus the sera did not react or reacted very weakly.

The experiments with luminescent immunoglobulin fractions against human immunoglobulins showed that antibodies against epithelial cells of the human thymus contained in the sera of patients with myasthenia gravis belong mainly to the G class and, to a lesser extent, the M class.

The results of a study of the reaction of the sera of patients with myasthenia with cells of the epithelial reticulum of the human thymus are given in Table 1. This table also gives data for the presence of antibodies against the cells of Hassall's corpuscles and the epidermis of the skin in the sera studied. In agreement with earlier findings [3] complete agreement was observed between the reaction of the sera with the cells of the Hassall's corpuscles and the epidermis. In addition, of 79 sera reacting with the cells of Hassall's corpuscles, 55 also contained antibodies against the cytoplasm of the cells of the epithelial reticulum. Comparison of these results with those of the reaction of the donors' sera showed that the percentage of sera reacting with the thymus epithelium is increased almost fivefold in myasthenia. Meanwhile the antibody titer also was increased from a normal 1:8-1:32 to 1:64-1:128.

Preliminary absorption of the sera of the patients with myasthenia and blood donors with thymus tissue homogenate and with a suspension of cells from the human embryonic and adult epidermis completely prevented their reaction with cells of the epithelial reticulum and Hassall's corpuscles of the human thymus and cells of the human epidermis. Absorption of the sera with tissue homogenates from other organs (liver, kidney, spleen, heart, brain) and erythrocytes of blood group AB did not affect the intensity of their reaction with the thymus epithelium. In connection with the results of absorption, a comparative study was made of the reaction of patients' sera containing antibodies against thymus epithelium with cells of the epidermis. On the sections of human skin the sera reacted with the cytoplasm and the perinuclear zone of the cells of the stratum granulosum and with the keratin of the stratum lucidum and stratum corneum. As a rule there was no reaction with the cells of the undifferentiated layers of the epidermis — the stratum basale and stratum spinosum. However, no regular agreement was found between the presence of antibodies against thymus epithelium in the serum and its reaction with antigens with a definite localization in the epidermal cells at this stage of the observations.

TABLE 1. Reaction of Sera of Patients with Myasthenia Gravis with Cells of Epithelial Tissue of Human Thymus and Epidermis of Human Skin

Source of serum	Number of sera studied	Number of sera reacting					
		with epithelial reticulum of thymus		with Hassall's corpuscles of thymus		with epidermis of skin	
		absol.	%	abs.	%	abs.	%
Patients with myasthenia gravis	90	55	56,6	79	82	79	82
Blood donors	50	5	10	30	60	30	60

The experiments thus showed that the serum of patients with myasthenia contains antibodies against antigen of the cells of the epithelial reticulum of the human thymus in a high proportion of cases and in an increased titer. The results of absorption of the sera with tissue homogenates from various organs and a suspension of cells of the human epidermis showed that this antigen is heteroorganic and belongs to the group of epidermal antigens of the thymus, i.e., antigens common with the cells of the epidermis. The blood of patients with myasthenia is known to contain lymphocytes sensitized to autologous thymus cells [8]. It was mentioned above that the serum of patients with this disease contains antibodies against antigens of myoid cells and cells of Hassall's corpuscles common with antigens of muscle tissue and skin epidermis, respectively. These results, like those of the present investigation, are evidence that in myasthenia gravis an immunopathological process takes place, directed against antigens of the tissues of the thymus, including against antigens of the heteroorganic structures of its epithelium.

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