DISCOVERY OF A SECRETORY COMPONENT IN MEMBRANE STRUCTURES OF HUMAN THYMUS

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It can now be considered established that an important feature of the thymus is that it contains heteroorganic antigens, i.e., antigens characteristic of certain highly specialized body tissues. One of the most intensively studied heteroorganic elements of the thymus is its myoid cells, whose cytoplasm contains antigens common with antigens of muscle tissues [10]. It has been shown that antigens common with those of the epidermis and other types of integumentary epithelium are represented in the epithelium of the thymus [2, 3, 7, 12, 13]. The thymus has been shown to contain cells which synthesize and secrete lactoferrin, a component of excretory fluids [6]. All these data are weighty evidence in support of the hypothesis of the instructive role of heteroorganic antigens, according to which they serve as the source of information on the structure of the body's own antigens during production of a state of natural immunologic tolerance to them [1, 3, 4].

This investigation is a continuation of others aimed at seeking heteroorganic antigens in the thymus. The foremost aim was to discover a secretory component (Sc) in the thymus which, like lactoferrin, is present in excretions from the epithelium of the mammary, salivary, and lacrimal glands, and also the epithelium of the intestine, bronchi, and certain other organs. One important function of Sc is that it binds molecules of serum IgA to form molecules of secretory IgA (SIgA).

EXPERIMENTAL METHOD

Unfixed frozen sections of human thymus (persons dying from trauma at the age of 10-22 years), tonsil (obtained during tonsillectomy), and human fetal thymus (22-24 weeks) were studied by the immunofluorescence method. SIgA were isolated from human colostrum by the method described previously [8]. Two preparations were used to detect Sc: 1) the globulin fraction isolated from the serum of rabbits immunized with SIgA and absorbed with human serum IgA treated with glutaraldehyde, labeled with fluorescein isothiocyanate (FITC); 2) antibodies isolated from rabbit antiserum against SIgA by means of immunosorbent SIgA, treated with glutaraldehyde, and then absorbed with serum IgA, also treated with glutaraldehyde. In direct immunofluorescence experiments sections of thymus and tonsil were incubated for 2 h at room temperature or 18 h at 4°C with FITC-labeled globulin fraction against SIgA, absorbed with serum IgA. In indirect immunofluorescence experiments sections of human thymus and tonsil were incubated for 18 h at 4°C with antibodies against Sc (600 μg/ml) and, after washing with buffered 0.85% NaCl solution (pH 7.4), they were treated with FITC-labeled antibodies of rabbit IgG. To detect cells of the plasma-cell series synthesizing IgA, sections of thymus and tonsil were treated for 18 h at 4°C with a labeled preparation against serum IgA or with a labeled preparation against SIgA, not absorbed with serum IgA.

EXPERIMENTAL RESULTS

Intensive fluorescence of membrane-like structures located in the subcapsular space and around the numerous tubular formations in the cortex and cortico-medullary zones of the gland was observed in sections of human thymus treated with anti-Sc preparations (Fig. 1: a-c). Membranes containing Sc located in the parenchyma of the thymus were distinguished by diversity of size and shape, and also by ability to form branches and numerous anastomoses with

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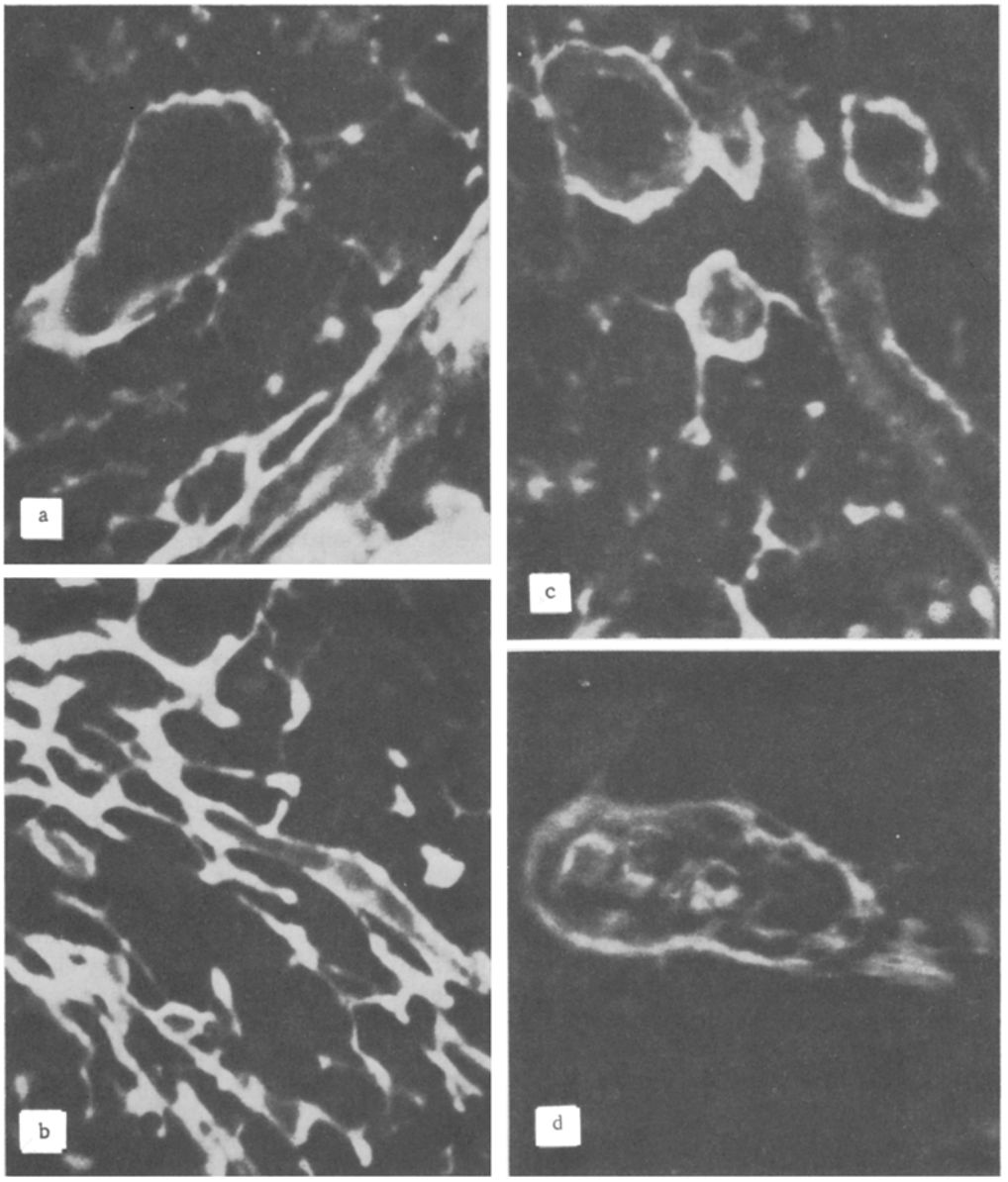

Fig. 1. Detection of Sc in membranous and tubular formations of human thymus and tonsil by immunofluorescence method: a) Sc in subcapsular membrane and around tubular formation in cortical zone of lobule; b) demonstration of Sc in cytoplasm of cells forming tubular structures of thymus, and in membranes adjacent to them; c) concentration of membrane structures containing Sc in parenchyma of thymus; d) Sc in cytoplasm of cells forming syncytium in human tonsils (more intensive fluorescence of cells of plasma-cell series is observed in the center of the syncytium).

one another (Fig. 1b). The dimensions of the tubular structures surrounded by membranes varied from tens to hundreds of microns. Examination of a transverse section showed that the cells forming these structures are arranged parietally, and accordingly a cavity is present inside the tubules (Fig. 1c). The cytoplasm of the cells contained a small quantity of Sc.

It will be noted that small tubules usually did not possess a structurally discrete membrane and were characterized by uniform diffuse fluorescence of their surface (Fig. 1c). Close connection was observed between membranes located beneath the capsule of the lobules, in the parenchyma of the thymus, and around the tubular formations, with changes from one into the other, so that they occupied the whole parenchyma of the cortical and the outer layers of the medullary zones of the lobules of the gland. Close to Hassall's corpuscles, right in the cen-

ter of the medullary zone, membranous and tubular structures containing Sc were rare. On treatment of thymus sections with preparations against serum IgA no fluorescence of cells of the plasma-cell series was observed.

In sections of human tonsils treated with anti-Sc preparations fluorescence of membranes surrounding tubular formations similar to those in the thymus was observed (Fig. 1e). However, fluorescence of the cytoplasm of the cells, which in most cases formed a syncytium, but sometimes were arranged parietally, was observed more frequently in the tubular formations of the tonsils than in the thymus. A system of branching membranes was not detected in the tonsils. By means of a preparation against SIgA, not absorbed with serum IgA, besides fluorescence of tubular structures, a reaction with the cytoplasm of cells of the plasma-cell series, located both in the parenchyma and in the territory of tubular formations containing Sc, also was observed in sections of the tonsils (Fig. 1d). Many cells of the plasma-cell series also were found in the parenchyma on treatment of sections of the tonsils with a labeled preparation against serum IgA, but the reaction with tubular structures was absent in this case.

In sections through human fetal thymus, treated with anti-Sc preparations, weak diffuse fluorescence of the surface of solitary tubular structures was observed in the cortico-medullary zone of the parenchyma. A system of membranes containing Sc was not found in the embryonic thymus. With the aid of the unabsorbed anti-SIgA preparation and globulin fraction against serum IgA, cells with coarsely granular cytoplasm, similar in their morphology to plasma cells, were found in sections of embryonic thymus. These cells were located in the lumen of blood vessels in the interlobular connective tissue, on its territory, from which they crossed into the parenchyma of the cortical zone. The same cells were found around the main vessels of the medullary zone of the thymus. They were not found close to structures containing Sc.

Many Sc, present in the system of membranous and tubular structures, were thus discovered in the human thymus by the immunofluorescence method. The existence of connections between membranes surrounding these structures and membranes located in the parenchyma of the thymus is evidence that all membranous structures, irrespective of their location, form a single system. Incidentally, the tubular formations around which membranes containing Sc were situated, resemble in their morphology blood vessels of small and medium caliber. This observation is in agreement with histological data showing the presence of a system of membranes around the vessels of the thymus, which surround them and apparently isolate them from the internal medium of the gland [9]. However, the possibility cannot be ruled out that this similarity between tubular structures and vessels is purely external in character and, in reality, they are glandular formations whose cells secrete Sc, which subsequently accumulates in the membrane system of the gland. This hypothesis is confirmed by observations of histologists showing the presence of glandular structures and cells morphologically similar to the goblet cells of the intestine and bronchi in the thymus, which produce a secretion similar in its staining properties to the mucin of the goblet cells of these organs [5, 9, 11]. On the whole, however, it must be admitted that the nature of the cells synthesizing Sc and the mechanisms of its accumulation in membranes of the thymus still remain unexplained and require further study.

The observations described above are evidence that the thymus has a number of features which distinguish it essentially from other Sc-producing organs, including the tonsils. Besides a well-developed system of membranes containing large quantities of Sc, an important feature distinguishing the thymus is that, because of the absence of cells synthesizing serum IgA in it, no SIgA is evidently formed, and the question therefore naturally arises of the function of Sc in the thymus. It will be noted that Sc is one of the heteroorganic antigens of the thymus and, together with other heteroorganic antigens of the thymus it evidently participates in the formation of natural immunologic tolerance to this component of the body. However, it is possible that heteroorganic antigens also perform other functions and take part in the differentiation of separate subpopulations of lymphocytes in the gland. In connection with the results of the present investigation it will be interesting to examine the question of whether Sc affects differentiation of thymus lymphocytes predetermined for repopulation of those lymphoid organs in which serum IgA is synthesized and SIgA is formed.

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INCREASED PERMEABILITY OF THE LYMPHOCYTE PLASMA MEMBRANE FOR MONO- AND BIVALENT CATIONS AND LOW-MOLECULAR-WEIGHT METABOLITES CAUSED BY MITOGENIC POLYANIONS

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The immunostimulating properties of polyanions such as polyacrylic acid (PAA) or dextran sulfate (DS) were described quite a long time ago. However, until recently the immunocompetent cells on which these agents acted, and how they acted, were unknown. Recently the writers determined the nature of lymphocytes which respond to PAA and described the detailed kinetics of their response with respect to parameters of cellular proliferation and differentiation [2, 3, 5, 6]. We are now investigating molecular changes taking place in the lymphocyte membrane under the influence of a polyanionic activator. Attention is being concentrated on functional systems in the plasma membrane which may be involved in the formation of the membrane-dependent signal that activates cell metabolism [4]. One such system is the ionic transport system. It consists of "channels" through which ions are transported along their concentration gradient, and of ion-transporting membrane enzymes, responsible for carrying ions against their concentration gradient, utilizing for this purpose the energy of the chemical bonds of ATP (for example, Ca^{++}-ATPase, Na^+, K^+-ATPase, etc.).

This paper describes the study of transmembrane flows of K^+ and Ca^{++} ions and also of nucleoside molecules before and after treatment of lymphocytes with immunostimulating doses of polyanions.

EXPERIMENTAL METHOD

In vitro cultures of splenic lymphocytes from (CBA × C57Bl)F_1 mice were used. To activate the lymphocytes mitogenic concentrations of PAA with molecular weight of 80,000 or 16,000 daltons [2] were added to the cultures. DS was added up to a final concentration of 200-500 μg/ml.

Permeability of the cell membrane for Ca^{++} was measured by a radio-indicator method. The isotope ^{45}Ca was used and was added to a suspension of lymphocytes (5×10^6 to 10×10^6 cells/ml), kept under optimal cultural conditions [2, 7]. Samples of 0.2-1.0 ml were taken from the suspension 1 h after addition of ^{45}Ca (final concentration 0.1 μCi/ml). Cells contained in the

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