IMMUNOFLUORESCENCE STUDY OF THYMUS CELLS SYNTHESIZING LACTOFERRIN

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The discovery of antigens in the cytoplasm of myeloid cells of the human and animal thymus that are common with components of muscle tissue [12] laid the foundations for the suggestion that the thymus contains antigens of several highly specialized tissues and organs, which "inform" the lymphocytes of the thymus about the structure of autoantigens and the process of development of a state of natural immunologic tolerance to them [2, 4]. Weighty support for this suggestion came from the results of investigations which showed that many antigens common with the epidermis and other epithelia of ectodermal origin are represented in the thymus [1-3, 5, 6, 15]. In this connection it is interesting to continue these investigations with a view to increasing our existing knowledge of the system of heteroorganic antigens of the thymus.

The object of the present investigation was to detect, by the immunofluorescent method, cells in the thymus which synthesize lactoferrin (LF), one of the main components secreted by cells of various organs (tonsils, salivary, mammary, and lacrimal glands, endometrium of the uterus, etc.). There is evidence that this protein is synthesized by epithelium and also by blood cells (evidently granulocytes), which penetrate into these organs from the blood stream [11, 13, 14].

EXPERIMENTAL METHOD

Antiserum against LF was obtained by immunizing rabbits with pure antigen isolated from human colostrum by gel filtration on Sephadex G-200 and ion-exchange chromatography on CM-cellulose [9]. To remove antibodies against serum protein, this immune serum was absorbed with normal human serum, treated with glutaraldehyde [10]. After absorption, the anti-LF-serum gave only one precipitation line in the gel precipitation test against protein of human colostrum, identical with LF, and it had a titer of 1:16-1:32.

The reaction of anti-LF-serum with structures in sections of the thymus, spleen, liver, and salivary gland of a human fetus (20-24 weeks) and adult and also with sections of the human tonsil, a polyp of the nasal mucosa, and the liver, kidney, heart and spleen (from subjects aged 14-22 years). Pieces of tissue from the test organs were frozen in petroleum ether, cooled in a mixture of acetone and dry ice (−70°C). Unfixed tissue sections (5-6 μ) obtained in a cryostat at −20°C were treated with anti-LF-serum (dilution 1:8) for 2 h at room temperature or for 18 h at 4°C, washed with buffered 0.85% NaCl solution, pH 7.2, and incubated for 45 min with antibodies against rabbit immunoglobulins, labeled with fluorescein isothiocyanate. The antibodies were isolated from antiserum with the aid of rabbit IgG, treated with glutaraldehyde [8, 10]. The specificity of the reaction of the anti-LF-serum with antigens in the sections was verified by preliminary absorption with pure LF, treated with glutaraldehyde [10].

EXPERIMENTAL RESULTS

On treatment of sections of the human thymus with anti-LF-serum by the indirect immunofluorescence method intensive fluorescence of the cytoplasm of many cells about 20 μ in diameter was observed (Fig. 1). Cells containing LF (LF-cells) were discovered in the in-

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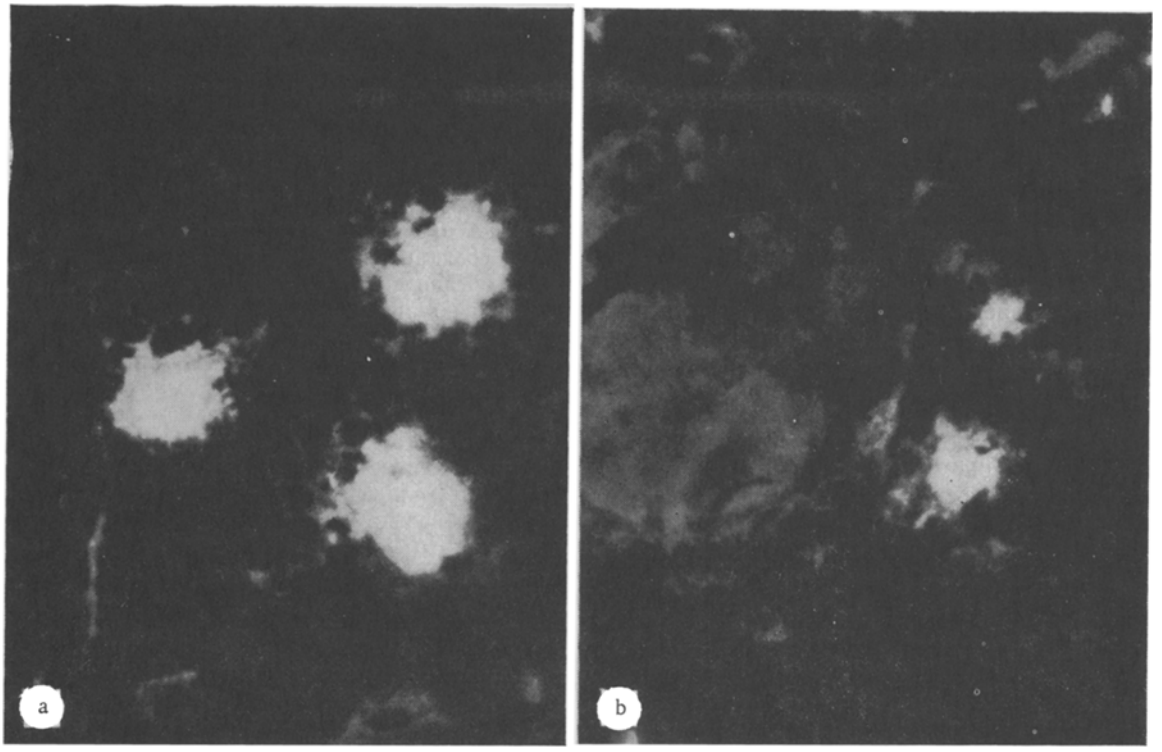

Fig. 1. Cells synthesizing LF in the human thymus: a) secretion of LF by thymus cells; b) attachments of LF-cell to a Hassall's corpuscle.

terlobular connective tissue, in the parenchyma of the cortical and medullary zones of the lobules of the thymus, in the lumen of the vessels, and in the perivascular spaces. Penetration of LF-cells was observed from the interlobular connective tissue into the subcapsular space, followed by their gradual migration into the parenchyma of the cortical zone. In the medullary zone LF-cells were observed to leave the vessels and penetrate into the parenchyma of the medullary layer. The characteristic morphological feature distinguishing LF-cells was the granular structure of their cytoplasm, especially of its peripheral zone. Furthermore, the LF-cells evidently possess secretory activity, for numerous granules became detached from their surface and were arranged along the borders of adjacent thymocytes and at a short distance from the LF-cells in the internal medium of the organ. Despite the fact that attachment of LF-cells to Hassall's corpuscles was observed comparatively rarely, nevertheless numerous granules containing LF could be seen on the territory of most of them (Fig. 1).

In sections of the tonsil LF-cells were found in much smaller numbers and they exhibited rather less secretory activity than in the thymus; moreover, they were predominantly located around blood vessels in the medullary zone of the follicles. The LF-cells of the tonsil differed from those of the thymus in the larger size of the granules in their cytoplasm, as a result of which their detachment during secretion, from the morphological point of view, resembled a process of disintegration of the cell into large clumps of material. Cells of this type containing LF were found in the mucous membrane of the nasal polyp.

In sections of the human salivary gland, LF-cells were found in the lumen and walls of blood vessels, in the perivascular spaces, and interstitial connective tissue, from which they penetrated to the territory of glandular structures and efferent ducts. Granules containing LF were seen above the cytoplasm of the epithelial cells and in the lumen of the ducts. In their morphology the LF-cells of the salivary gland were similar to LF-cells of the thymus, but they were less numerous in the former and evidently possessed rather less secretory activity.

On treatment of sections of the human liver with anti-LF-serum numerous diffusely fluorescent cells about 10 μ in diameter could be seen in the parenchyma of the organ. Single, very tiny granules, containing LF, were detached from their surface. Similar cells, but in much smaller numbers, were seen in sections of the spleen; solitary LF-cells, similar

in their morphology with the LF-cells of the liver and spleen, but giving more intensive fluorescence, were found in individual glomeruli of the kidney and in the interstices of the myocardium.

Numerous cells containing LF were found in sections of the embryonic human thymus. In this case many cells measuring about 15 μ in diameter, with a coarsely granular cytoplasm, were seen in the lumen of the vessels in the interlobular connective tissue and in the medullary zone of the lobules of the gland. As they left the vessels and penetrated into the parenchyma of the cortical and medullary zone these cells grew in size to 20 μ the granules became smaller but increased correspondingly in number, as a result of which the cells came to resemble morphologically the LF-cells of the adult human thymus. LF-cells in the embryonic thymus showed high secretory activity, reflected in the presence of numerous granules around the LF-cells. In the human fetal liver and spleen there were solitary cells similar in morphology to the LF-cells of the adult human organ. In sections of the human fetal salivary gland, no cells synthesizing LF could be found in this period of embryonic development.

It should be noted that most immune sera, like the sera of unimmunized rabbits, reacted with the cytoplasm of cells of the epithelial reticulum and Hassall's corpuscles of the human thymus, with cells of the efferent ducts of the salivary glands and of the nasal mucosa, with the sarcoplasm of myocardial muscle fibers, and with the cytoplasm of parenchymatous cells of the liver. However, only sera of animals immunized with this antigen reacted with LF-cells. Preliminary absorption of the anti-LF-sera with pure LF treated with glutaraldehyde completely inhibited their reaction with the LF-cells of all organs studied but did not affect the reaction with other antigens of these organs.

The results thus indicate that the human thymus contains many cells which synthesize LF and secrete it into the internal medium of the organ. The population of LF-cells of the thymus also have certain features which distinguish it significantly from the other organs studied. First, the number of LF-cells in the thymus and their secretory activity are appreciably greater than in organs producing this protein, such as the salivary gland and tonsil. Also, according to the results of this investigation, during embryonic development LF-cells appear sooner in the thymus than in the salivary gland; their number and functional activity in the embryonic thymus, moreover, not only differ from those in the adult human thymus, but may perhaps even surpass them. In this respect the LF-cells resemble another heterogeneous structure of the thymus, namely the myeloid cells, the number and secretory activity of which are significantly greater in the embryonic period than in the adult human thymus [7]. These observations, taken together, suggest that besides its other known functions — bacteriostatic and binding bivalent ferrous ions — the LF in the thymus also performs the function of a heteroorganic antigen.

To conclude, the results of these observations agree with those of Masson et al. [14] and are evidence that LF is synthesized in all the organs which we studied and, in particular, in the thymus, tonsils, and salivary gland, not by the epithelium of these organs but by cells of hematogenous origin, i.e., cells which migrate into these organs from the blood stream. To elucidate the causes of disagreements regarding the histogenesis of LF-producing cells and, in particular, to determine the ability of the epithelium of the thymus to synthesize this protein, further investigations are required.

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ORGAN-SPECIFIC BETA-GLOBULIN OF THE HUMAN PROSTATE DURING TUMOR GROWTH

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The study of the antigenic structure of human organs and tissues is an urgent problem at the present time because of the needs of medical practice and, in particular, of oncology. The characteristics of organ-specific antigens occupy a central position in this problem because, as specific markers of a tissue, they enable the degree of differentiation, the functional maturity and, in some cases, commencing malignant transformation of a tissue to be determined.

The study of the antigenic structure of the human prostate has so far been confined mainly to investigation of only one antigen, namely organ-specific acid phosphatase, the physicochemical properties, structure, and diagnostic importance of which have been characterized in detail [7-9].

While engaged on the study of the antigenic structure of the prostate, besides acid phosphatase we have also identified another organ-specific antigen, which does not possess phosphatase activity and migrates during immunoelectrophoresis in agar in the beta-globulin zone.

The object of this investigation was to study the immunochemical and physicochemical properties of prostatic organ-specific beta-globulin, its tissue localization, and its behavior during tumor growth.

EXPERIMENTAL METHOD

Saline extracts of fetal, definitive, and tumor tissues of the prostate, prepared in Tris-glycine buffer, pH 8.6, in the ratio of 1:3 (w/v), were used. The control group consisted of extracts of fetal and definitive tissues of various human organs prepared in the same way.

Prostatic beta-globulin (PBG) in the tissues was identified by immunoelectrophoresis [1] and double immunodiffusion in agar [5]. The PBG concentration in the tissues and biological fluids was determined by immunodiffusion titration in agar with a standard test system, the sensitivity of which was 0.3 mg %.

PBG isolated from a saline extract of normal prostate and purified by the writers' own method,* was used as the test antigen.

The test antisera were obtained by immunizing rabbits in the usual way both with pure PBG and with saline extracts of definitive prostate mixed with Freund's complete adjuvant. Only monospecific antisera were used in the work; polyvalent antisera were absorbed with

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