DISTRIBUTION OF A CROSS-REACTING ANTIGEN COMMON TO EPITHELIUM AND GROUP A STREPTOCOCCI IN HUMAN AND ANIMAL TISSUES

L. V. Beletskaya, É. V. Gnezditskaya, I. M. Lyampert, N. A. Borodiyuk, I. I. Rassokhina, and T. A. Danilova

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The location of a cross-reacting antigen common to streptococci and human and animal tissues was studied by the indirect immunofluorescence method, using pure antibodies against the polysaccharide of group A streptococci and pure antibodies against rabbit immunoglobulins, labeled with fluorescein isothiocyanate, in epithelial cells of ectodermal origin, namely the skin, salivary ducts, mucous membrane of the mouth, esophagus, lower part of the rectum, and cervix uteri, and in elements of the epithelium of the thymus. The discovery of the cross-reacting antigen is interesting in connection with the study of mechanisms of development of immunopathological processes in man connected with streptococcal infection and with the investigation of the structure, function, and pathology of the thymus.

KEY WORDS: streptococcus; thymus; epithelium; cross-reacting antigen; autoimmunity.

Previous investigations have shown that antibodies against the polysaccharide of group A streptococci (A polysaccaride), as tested by the indirect immunofluorescence method, react with epithelial elements of the skin and thymus of man and animals [4]. These reactions are found in all tissues so far studied and they are unconnected with isoantibodies. Antibodies reacting with antigen of epithelial elements have been shown to be directed toward the terminal determinant specific for A polysaccharide which, as some workers [7] have found, contains β-N-acetylglucosamine. The specificity of the reaction of the antibodies with epithelial tissue antigen has been demonstrated by inhibition by A polysaccharide and by synthetic N-acetylglucosamine (with β rotation). The study of the localization and distribution of cross-reacting (CR) antigens in human and animal tissues is important in connection with the study of the mechanisms of tissue damage in autoimmune processes. Production of pure antibodies against individual antigenic determinants has presented wide opportunities for precise investigation of the localization of such determinants in the tissues and also for the study of certain aspects of histogenesis of some epithelial tissues, including the epithelium of the thymus.

In the investigation described below the method of indirect immunofluorescence with pure antibodies against the polysaccharide of group A streptococci was used to study the distribution of CR antigen in the epithelial tissues of some species of mammals.

EXPERIMENTAL METHOD

Preparations of pure antibodies against group A streptococcal polysaccharide were used. Serum containing antibodies against A polysaccaride were obtained by immunizing rabbits with a heat-killed cul-

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology. Laboratory of Biochemistry, Institute of Rheumatism, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 212-214, February, 1976. Original article submitted February 5, 1975.

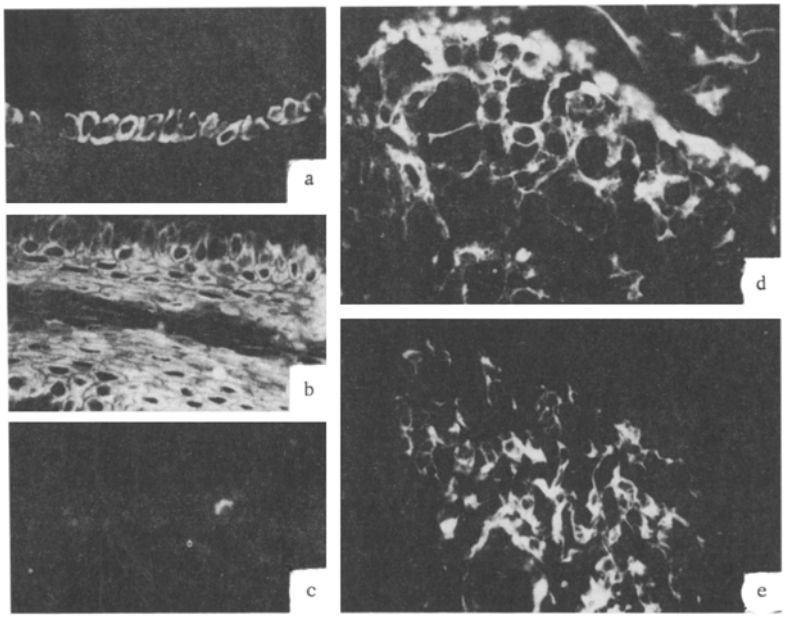

Fig. 1. Detection of CR antigen in sections of various human and animal tissues; a) human (blood group 0) skin: localization of CR antigen in peripheral zone of cytoplasm in cells of basal layer of epithelium; b) section through cervix uteri of a mouse: fluorescence of cytoplasm of cells in all layers of epithelium; c) section through mouse cornea: no reaction; d) section through human thymus removed at thymectomy: reaction in peripheral zone of cytoplasm and processes of stellate cells of epithelial reticulum; e) section through guinea pig thymus removed 24 h after irradiation (1000 R): reaction with cytoplasm of cells of peripheral zone of Hassall's corpuscles and of epithelial reticulum surrounding corpuscles. Indirect immunofluorescence method. Objective 40× (water immersion), ocular 3× (homal).

ture of group A streptococci (type I), treated with pepsin by Krause's method [6]. The A polysaccaride was isolated by Fuller's method [5].

Pure antibodies against the polysaccaride were obtained by the method of Osterland and Krause [8] by decomposing the precipitate containing the antigen–antibody complex in an acid medium, followed by chromatography on Sephadex G-100. The preparation of pure antibodies contained from 300 to 750 μg protein/ml. To remove antibodies against other streptococcal antigens the sera were first absorbed with a culture of group A streptococci of a variant containing polysaccharide without the terminal determinant (β-N-acetylglucosamine) [7]. The method of isolation and labeling of pure antibodies against rabbit immunoglobulins with fluorescein isothiocyanate was described previously [3].

Most of the investigations were carried out on mouse tissues. Organs from rats, guinea pigs, and rabbits, bovine organs, and human organs (including those of a 12-20-week fetus) from persons of different blood groups also were used. Tissues of the skin, eye, salivary glands, mucous membrane of the mouth, esophagus, stomach, rectum, large intestine, urinary bladder, uterus, and cervix uteri, and from the thymus and other organs were investigated. Sections 4-5 μ in thickness were cut in a cryostat (−20°C) from frozen unfixed tissue. Antibodies against streptococci polysaccharide were applied to unfixed sections for 45 min at room temperature, after which the sections were washed in 0.85% sodium chloride solution (pH 7.0) with phosphate buffer for 10 min, treated under the same conditions with pure antibodies or with serum against rabbit immunoglobulins labeled with fluorescein isothiocyanate, and after rinsing for 10 min they were mounted in neutral glycerol.

EXPERIMENTAL RESULTS

Tests of the preparation of antibodies against A polysaccharide on sections of human, mouse, rat, guinea pig, and rabbit skin tissues revealed bright fluorescence of the peripheral zone of cytoplasm in cells of the basal layer of the stratified squamous epithelium (Fig. 1a).

A reaction also was observed with the cytoplasm of the cells of the appendages of the skin. The cells of the more highly differentiated layers of the epidermis, of the dermis itself, of the striated muscle adjacent to the skin, the blood vessels, nerve trunks, and other structures in the skin tissues (fibers and ground substance) did not react with antibodies against A polysaccharide. Investigation of sections through the epithelium of other organs revealed specific fluorescence in the cytoplasm of cells of the basal layer of the stratified squamous epithelium of the mucous membrane of the sclera, the mouse, the salivary ducts, esophagus, and lower third of the rectum. The mouse esophagus was investigated at all levels, including the anterior part of the stomach, which also gave a positive reaction.

Fluorescence was observed in sections through the mucous membrane of the cervix uteri in the cytoplasm of cells in all layers of the epithelium (Fig. 1b). Epithelial cells of the cornea and of the mucous membrane of the stomach, the proximal parts of the rectum, the large intestine, urinary bladder, and the body and tubes of the uterus did not react with antibodies against streptococcal polysaccharide (Fig. 1c). As well as the tissues listed above, positive reactions were obtained with the epithelium of the thymus from man and animals (guinea pig, rabbit, rat, mouse).

Investigation of sections through the animal thymus revealed specific fluorescence in the cytoplasm of peripheral elements of Hassall's corpuscles and of individual epithelial cells surrounding the corpuscles. In sections through tissues of the human thymus, besides the cells composing the thymic corpuscles and elements of the medullary zone, a reaction also was observed with antigen in the cytoplasm of the cells of the basal epithelium of the cortical zone, located at the periphery of the lobule.

Investigation of sections through tissues of the human thymus (obtained at thymectomy on a patient with myasthenia gravis), with a sharply reduced number of lymphocytes, showed that the antibodies reacted with antigen located in the peripheral zone of the cytoplasm and processes of the stellate cells of the thymus, forming the epithelial reticulum, and cells of the cortical (basal) epithelium of the lobule (Fig. 1d). In the study of sections of the guinea pig thymus removed 24-72 h after irradiation (1000 R), as a result of which practically all the lymphocytes disappeared from the thymus but the epithelial tissue remained, fluorescence was observed only in cells of the epithelial bands in the center of the lobule (Fig. 1e). Antigens in cells of the cortical epithelium of the guinea pig thymus did not react with antibodies against A polysaccharide. In control experiments to test rabbit immunoglobulins containing antibodies against dinitrophenyl and also immunoglobulins isolated from normal rabbit serum (with the same protein content as the preparation of antibodies against A polysaccharide) fluorescence of the epithelial cells was absent. Inhibition of antibodies against A polysaccharide by N acetylglucosamine (a synthetic preparation with β rotation, 80 mg/750 μg antibody protein) inhibited the reaction of the antibodies with the epithelial elements of the tissues.

The experiments thus showed that a CR determinant, common to the polysaccharide of group A streptococci and antigens of the cells of some epithelial tissues, is found in man and in various species of animals. This CR antigen is located in the peripheral zone of the cytoplasm of the epithelial cells of the skin and of the mucous membranes of the mouth, esophagus, salivary ducts, the lower part of the rectum, and the cervix uteri. These tissues share a common ectodermal origin. The positive reactions obtained with elements of the epithelial recticulum of the thymus point to its ectodermal nature. The results are interesting from the point of view of explaining the mechanisms of development of tissue pathology in autoimmune processes in man connected with streptococcal infection.

Another promising field is the use of antibodies against this CR antigen as a label with which to study the histogenesis of epithelial tissues, including the epithelium of the thymus.

The discovery of CR antigen in the thymus is particularly interesting, because this organ is the target organ in several autoimmune processes, including in rheumatic fever [1, 2, 9].

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