AUTOANTIBODIES AGAINST THE COMMON ANTIGENIC DETERMINANT OF GROUP A STREPTOCOCCAL POLYSACCHARIDE AND EPITHELIAL CELLS OF MAMMALIAN THYMUS AND SKIN

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UDC 576.851.214.097.2

A cross-reacting (CR) antigen, group A streptococcal polysaccharide and antigen of mammalian thymus and skin, was studied. The CR antigen was found in all adult human and embryonic tissues regardless of blood group, in all animals of different species studied, and in the tissues of rabbits immunized with streptococci and producing antibodies against A-polysaccharide. The results indicate that antibodies developing against the CR-determinant of A-polysaccharide and epithelial tissues belong to the class of autoantibodies. The reaction of these autoantibodies with the CR antigen is probably one cause of the development of autoimmune thymitis in rheumatic fever.

KEY WORDS: autoantibodies; cross-reacting antigens; streptococcus.

Previous investigations showed that antibodies against streptococcal group A polysaccharide (A-polysaccharide) react with epithelial cells of the skin and thymus of man and animals when tested by the indirect immunofluorescence (IF) method [5].

Investigations on human and animal tissues were carried out with pure antibodies against A-polysaccharide isolated from streptococcal antisera. The use of immunodiffusion methods and experiments with adsorption and inhibition by different streptococcal antigens and synthetic N-acetylglucosamine showed that the antibodies were directed only against the specific determinant for A-polysaccharide [4, 5].

The specificity of the reactions with epithelial cells also was confirmed by inhibition of these reactions by A-polysaccharide or by synthetic N-acetylglucosamine. These findings indicate the presence of a cross-reacting (CR) determinant in A-polysaccharide and in mammalian epithelial cells [3, 5]. A true CR-antigen is also found in the epithelial cells of the mucous membrane of the sclera of the eye and other epithelial tissues of ectodermal origin [3].

Usually autoantibodies arise during immunization with CR antigens of microbial origin only if these antigens are not isoantigens and correspond to organ- or tissue-specific antigens. The soundest evidence of the autoimmune nature of antibodies arising during immunization by CR antigens of microorganisms is the discovery of the corresponding CR antigen in the tissues of the organism to be immunized [6].

The object of the present investigation was to determine whether antibodies arising against A-polysaccharide and reacting with epithelial tissue cells belong to the category of autoantibodies. To solve this problem it was necessary to determine whether the CR antigen of the epithelium is a tissue-specific antigen or isoantigen. The presence of the corresponding CR-determinant in the tissues of immunized animals producing antibodies against the CR-determinant of A polysaccharide had also to be investigated.

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, 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. 5, pp. 570-572, May, 1976. Original article submitted May 20, 1975.

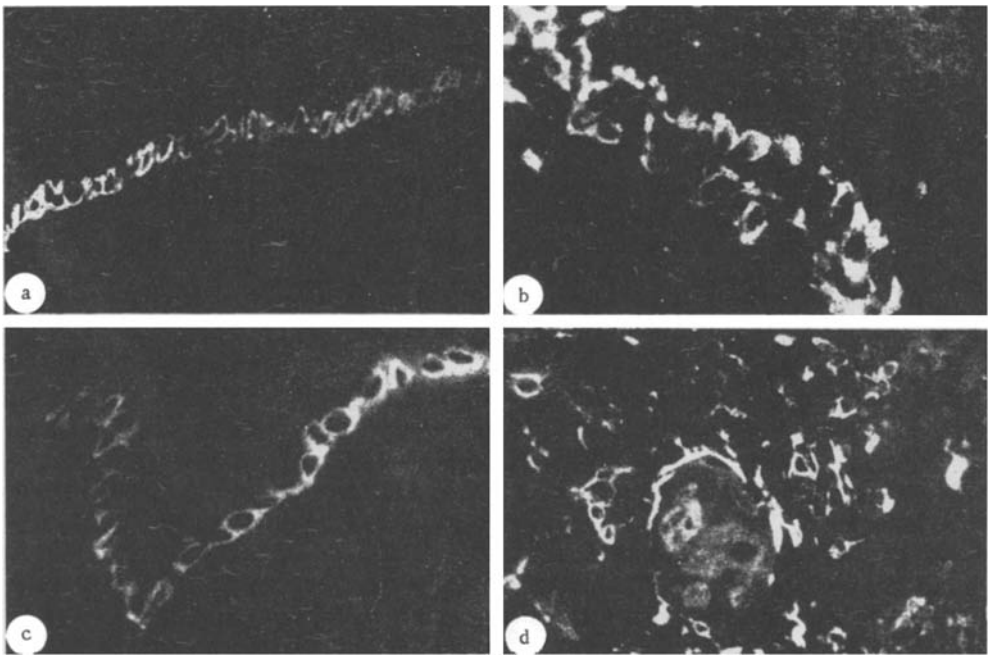

Fig. 1. Reaction of antibodies against group A streptococcal polysaccharide and epithelial cells of skin and thymus: a) section through skin of human fetus (age 20 weeks, blood group O); reaction with antigen of peripheral zone of cytoplasm in cells of basal layer of epidermis; b) tissue section of guinea pig thymus, reaction with epithelial structures of peripheral zone of Hassall's corpuscle; c) tissue section through adult human thymus; reaction with epithelial structures of basal layer of cortical zone of thymus; d) section through skin of rabbit producing antibodies against A-polysaccharide; reaction with same structures as in Fig. 1a. Objective 40 ×, ocular, homal 3 ×.

EXPERIMENTAL METHOD

Rabbits were immunized with a culture of group A type 1 streptococcus grown and subcultured on a meat medium or medium with casein hydrolysate and treated with pepsin. The A-polysaccharide was prepared by the formamide method from cell walls. The sera were preliminarily adsorbed with a culture of an A-variant whose polysaccharide did not contain the specific determinant for A-polysaccharide. Pure antibodies were isolated from the adsorbed sera by decomposition of the antigen–antibody complex and chromatography on a Sephadex G-100 column. The isolated antibodies (5-10 mg protein/ml) were verified by the precipitation test (PT) in gel against A-polysaccharide and other streptococcal antigens. Other experiments were carried out to study inhibition of the reaction of antibodies with A-polysaccharide by means of A-polysaccharide (200 μg polysaccharide/ml) or of synthetic N-acetylglucosamine (β rotation, 80 mg/ml).

The methods of preparing and checking the pure antibodies against A-polysaccharide were described more fully previously [4]. For tests by the indirect IF method preparations of pure antibodies were used in dilutions containing from 300 to 750 μg protein/ml. Antibodies against A-polysaccharide were tested on tissue sections of the thymus (19 sections) and skin (18 sections) from human adults and embryos (age 15-20 weeks). Thymus was obtained from six persons with blood group O, and skin from four such individuals. Tissue sections of the thymus and skin of 24 animals (rabbits, guinea pigs, rats, mice), including rabbits immunized with group A streptococcus, also were studied. Antibodies against the specific determinant for the A-polysaccharide were determined by the PT test in gel in the serum of these animals [4].

To test antibodies against A-polysaccharide in sections obtained from frozen, unfixed tissues, the indirect IF method with pure antibodies against rabbit IgG, labeled with fluorescein isothiocyanate [4], was used. In the control experiments the reaction between antibodies and tissue antigens was inhibited by A-polysaccharide or N-acetylglucosamine in the doses specified above. During adsorption of the antibodies by red cells of blood group AB, 0.25 ml of wet residue of red cells was added to 1 ml antibodies. Ig isolated from normal rabbit sera and from sera containing antibodies against dinitrophenyl (DNP)* also were tested as a control.

*These immunoglobulins were kindly presented by I. S. Tarkhanova.

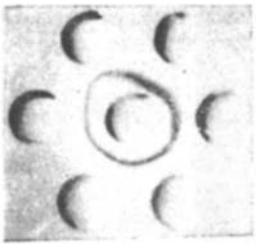

Fig. 2. Reaction of serum from a rabbit immunized with streptococci with A-polysaccharide. Central well contains serum, peripheral wells contain A-polysaccharide (from 1 mg to 12 μg/ml).

EXPERIMENTAL RESULTS

Tests of antibodies against A-polysaccharide by the IF method in sections of adult and embryonic human and animal skin gave reactions in the peripheral zone of the cytoplasm of cells from the basal layer of the epidermis (Fig. 1a). In sections of the animal thymus reactions were observed with the cytoplasm of peripheral elements of the Hassall's corpuscles and epithelial cells surrounding these structures. In tissue sections of adult and embryonic human thymus, besides reactions with the above-mentioned structures, reactions also were found with the epithelial cells of the cortical zone of the thymus lobules (Fig. 1d, b).

Tests of sections of skin and thymus of human adults and embryos and animals were positive for all tissues studied, including sections of tissues from individuals with blood group O. Antibodies against A-polysaccharide isolated from the sera of animals immunized with streptococci grown on different nutrient media gave identical results. Tests of thymus and skin tissues from rabbits immunized with streptococci by the IF method gave reactions with the same structures as in unimmunized animals (Fig. 1c). Antibodies reacting in the PT in gel with A-polysaccharide were found in the sera of immunized animals (Fig. 2).

Negative results were obtained in human and animal tissues when testing immunoglobulins isolated from normal sera and animal sera containing antibodies against DNP.

Adsorption of antibodies against A-polysaccharide by group AB red cells had no effect on the reaction of the antibodies with the tissue structures. Meanwhile, either A-polysaccharide or N-acetylglucosamine inhibited these reactions.

The study of antibodies against A-polysaccharide by the IF method thus gave reactions with the same structures of the skin and thymus as were described previously [3, 5].

The positive reactions obtained with the use of all specimens of tissues from man and animals of different species and the independence of these reactions of blood group in man are evidence that the CR antigen under investigation belongs to the group of tissue-specific antigens. These results, and also the discovery of CR antigens in rabbits producing antibodies against A-polysaccharide, indicate that antibodies against true CR antigen are autoantibodies.

The development of thymitis has been described in a number of articles on rheumatic fever and other autoimmune (AI) processes. In connection with the discovery of antibodies against A-polysaccharide in the sera of patients with rheumatic fever, the possibility must be considered that one cause of the development of AI-thymitis is the reaction of these antibodies with thymus CR antigen. The presence of heteroorganic antigens (organ-specific antigens of several organs found in the thymus [1, 2, 7, 10]), in the opinion of Burnet [7] and others [1, 2], contributes to the development of tolerance toward its own tissue antigens. At the same time, when the pool of thymocytes in the cortical layer is exhausted, immunocompetent cells can still be found in the medullary zone of the thymus. Lymphocytes reacting in vitro with tissue antigens in a syngeneic system have also been found in the thymus [9].

Immunodepressants (ID) found in the thymus [8] are evidently the factors which under normal conditions prevent a reaction between the immunocompetent cells of the thymus and tissue antigens. It is known that ID exert a co-tolerogenic effect during contact between T cells, maturing in the thymus, and heteroorganic antigens. Disturbance of the ID function of the thymus, which may arise in AI-thymitis, must evidently facilitate the development of cellular AI reactions, above all in the thymus itself, through the reaction between the maturing T cells and heteroorganic antigens. Loss of the ID function of the thymus in mice of the NZB strain during the development of a spontaneous AI process [11] is in full agreement with this suggestion.

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AGE CHANGES IN IMMUNE RESPONSE OF RATS TO REPEATED INJECTIONS OF DIFFERENT DOSES OF SHEEP'S RED CELLS

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UDC 612.67.017.1

The number of antibody-forming cells and the serum antibody titers were determined in adult and old rats after tenfold immunization with "small" ($4 \cdot 10^8$ cells) and "large" ($4 \cdot 10^{10}$ cells) doses of sheep's red cells. The antibody (hemolysin and hemagglutinin) titers in the animals of both age groups were found to be either similar in magnitude or higher in the younger adult rats (for hemolysins, in the case of injection of the "large" dose of antigen). The number of direct plaque-forming cells in the spleen of the old animals was greater than in the young adults at all times of immunization, but the number of indirect plaque-forming cells was greater only at the end of immunization. The results are evidence of differences in maturation of the immune response in animals of different ages.

KEY WORDS: immune response; age differences.

For a long time the view has been firmly held that with age the antibody-forming ability of the organism diminishes, in connection with a decrease in the number of potential antibody-forming cells [3, 11, 13, 18]. Recently, however, a definite decrease during aging has been found only with respect to the primary immune response [4, 6, 14, 16], whereas the secondary response shows a much smaller decrease or, in general, shows no change [7, 9, 15, 17]. There is evidence that the change in antibody-forming ability in old age may be due not so much to a deficiency of antibody-forming cells as to a disturbance of regulatory mechanisms [4, 6, 8]. This hypothesis seems perfectly probable, for histological investigation of lymphoid tissue in hyperimmune rats showed a well-marked cellular response in old animals [2]. The writer has also shown that old animals can produce antibodies either more or less actively than younger adults, depending on the dose and duration of injection of the antigen [1].

The object of the present investigation was to study the possible mechanism of differences in the state of antibody formation in old animals under different experimental conditions.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats of two age groups: adult (8-10 months) and old (24-26 months).

Antigen – sheep's red cells – was injected intraperitoneally ten times (at intervals of seven days) in doses of $4 \cdot 10^8$ cells (the "small" dose) and $4 \cdot 10^{10}$ cells (the "large" dose). This particular scheme of im-

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