ANTIBODIES REACTING WITH THYMUS AND SKIN EPITHELIUM AND ANTIBODIES TO CELL NUCLEI DURING IMMUNIZATION WITH GROUP A STREPTOCOCCAL POLYSACCHARIDE CONJUGATED WITH SYNTHETIC POLYELECTROLYTES

E. V. Ryzhikova, A. V. Nekrasov, I. M. Lyampert, V. Yu. Kolesnikova, N. A. Borodiyuk, E. Yu. Pyt'eva, and R. M. Khaitov

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Cross sections have been described between group A streptococcal polysaccharides (A-PSC) in the epithelium of the skin and thymus [3, 11]. As a result of isolation of the antibodies by affinity chromatography it was found [1] that only some antibodies to A-PSC, namely high-affinity antibodies, will react with epithelium. It has been suggested [11] that antibodies arising during streptococcal infection to a cross-reacting (CR) determinant of A-PSC can injure the epithelial tissue of the sinus and induce immunoregulatory disturbances leading to the development of an autoimmune process.

The study of the role of antibodies to the Cr-determinant of A-PSC during the autoimmune process is made more difficult by the fact that A-PSC is a nonimmunogenic hapten. In addition, during immunization with whole group A streptococcal cells, antibodies to A-PSC cross-reacting with epithelium can be obtained only in certain animals and in small quantities [1, 11].

Recent investigations have shown that synthetic polyelectrolytes (PEL) and, in particular, copolymers of acrylic acid (AA) and N-vinylpyrrolidone (N-VPD), are powerful stimulators of the immune response. Conjugates of synthetic PEL with haptens are able to induce a marked hapten-specific immune response [4, 5].

Conjugates of A-PSC with synthetic PEL were used in the present investigation to obtain an immune response to the common antigenic determinant of A-PSC and epithelial tissue.

EXPERIMENTAL METHOD

Purified A-PSC was obtained and isolated by the formamide method from a culture of group A streptococcus treated with pepsin [8]. Conjugates of A-PSC with copolymers of AA and N-VPD of equimolar composition were used [6]. Primary amino groups were introduced by an acetylation reaction by the carbodiimide method. Copolymers containing 20% of modified carboxyl groups were used. The conjugates were synthesized by the triazine method. In a neutral medium (water–DMFA) 2-amino-4,6-dichloro-symm-triazine was bound with the polysaccharide, after which, in an alkaline medium (pH 9.5-10.0) it reacted with the amino group of the copolymer. The conjugate was isolated by gel-chromatography, dialyzed, and lyophilized [6]. The content of A-PSC in the conjugate was determined from the quantity of rhamnose [9]. Conjugate No. 1 (in 100 μg) contained 10 μg of A-PSC, whereas conjugate No. 2 (in 100 μg) contained 24 μg of A-PSC. The immunization scheme included two cycles of intraperitoneal injections with an interval of 4 weeks between cycles [2]. The first cycle consisted of four, the second of five injections, given once a week. Female BALB/c mice weighing 16-18 g were immunized with each conjugate (three groups, with 10 animals in each group). The conjugates were injected in a dose of 10 or 20 μg of A-PSC per mouse at each injection (1st and 2nd groups, respectively),

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 6,pp. 707-710, June, 1987. Original article submitted May 6, 1986.

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or in doses of 10, 20, 30, and 40 μg during the 1st to the 4th injections in the first cycle of immunization, and thereafter in a dose of 50 μg of A-PSC per mouse in the second cycle of immunization (group 3). PEL or A-PSC with Freund's complete adjuvant were injected in the same doses into control animals. One of the control groups of mice was immunized with a culture of group A streptococcus treated with pepsin [7]. Blood was taken from the animals on the 5th-6th day after immunization.

Antibodies to A PSC were determined in the sera of the immunized mice by enzyme immunoassay (EIA), using 96-well plates of Soviet manufacture, sensitized with a suspension of a culture of group A streptococcus treated with pepsin ($2.5 \cdot 10^8$ microbial cells/ml) [2]. Orthophenylenediamine was used as the substrate. Antibodies to mouse immunoglobulins, labeled with peroxidase, were used (the antibodies were obtained from the Laboratory of Immunologic Diagnosis, Director Professor K. L. Shakhanina, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). The reactions were read on a photometer (from Titerteck, England).

To determine antibodies reacting with epithelial cells the indirect immunofluorescence test (IIFT) was used, as described previously [11], with antibodies to mouse immunoglobulins labeled with fluorescein. Sera of immunized mice were tested on unfixed frozen sections through the skin over a joint of 18-week human fetuses (blood group O) and the skin of the lip of BALB/c mice, on sections through the thymus of persons dying from trauma (blood group O), and also on sections of thymus and liver tissue from BALB/c mice. To inhibit reactions with tissue antigens, A-PSC was used (2 mg of A-PSC to 0.2 ml of serum, diluted 1:8). The serum was incubated with A-PSC for 2 h at 37°C and for 18 h at 4°C. The preparations were examined under the ML-2 luminescence microscope with a 40× objective. A homal 3 ocular was used for photography.

EXPERIMENTAL RESULTS

During determination of antibodies to A-PSC by EIA, in all three groups of animals immunized with conjugate No. 1 negative results were obtained after the first cycle of injections. Antibodies were found in titers of 1:200-1:400 in animals of the 2nd and 3rd groups after the second cycle of immunization. All sera of animals immunized with conjugate No. 1 gave negative reactions when tested with epithelial tissue of human and mouse skin and thymus in the IIFT.

After the first cycle of immunization with conjugate No. 2 antibodies to A-PSC were not found in the animals of group 1 by EIA, or they were found in low titer (under 1:200). In the 2nd and 3rd groups these antibodies were found in titers of 1:400-1:800. After the first three injections of the second cycle of immunization antibody titers to A-PSC in the animals of group 1 increased to 1:400-1:800, whereas in the sera of individual animals of groups 2 and 3 they increased to 1:1600. Further immunization was accompanied by a fall of antibody titers to A-PSC in animals of all three groups.

When the sera of mice immunized with conjugate No. 2 were tested by the IIFT, antibodies to epithelial tissue of the skin were not found in the animals of group 1 after the first cycle of injections. In mice of groups 2 and 3, under these circumstances, antibodies reacting with the stratum basale of the epidermis of human and mouse skin were found in mice of groups 2 and 3 in titers of 1:8-1:32 (Fig. 1a). After two or three injections in the second cycle of immunization antibodies to the stratum basale of the skin were found in a titer of 1:32 in all groups of animals immunized with conjugate No. 2. On further immunization the intensity of reactions of the sera with the stratum basale of the skin fell somewhat. At the same time, after four or five injections of the second cycle of immunization with conjugate No. 2 the sera reacted strongly with layers of the skin epithelium above the stratum basale, giving rise to diffuse or perinuclear fluorescence of the cytoplasm of the epithelial cells. Strong reactions also were observed with cell nuclei on sections through epithelial tissues and liver tissue (Fig. 1b).

After the end of the second cycle of immunization antibodies to nuclei were discovered in a titer of up to 1:128 in all sera tested in animals of groups 2 and 3 and in two animals of group 1. It was shown that A-PSC inhibit the reaction with the stratum basale of the epithelium of the skin. Reactions with cytoplasm were preserved in layers of the skin epithelium above the stratum basale and in cell nuclei. In the control groups of animals, immunized with copolymers or A-PSC with Freund's complete adjuvant, antibodies to A-PSC and antibodies reacting with different structures of epithelial tissue and with cell nuclei were not found. On

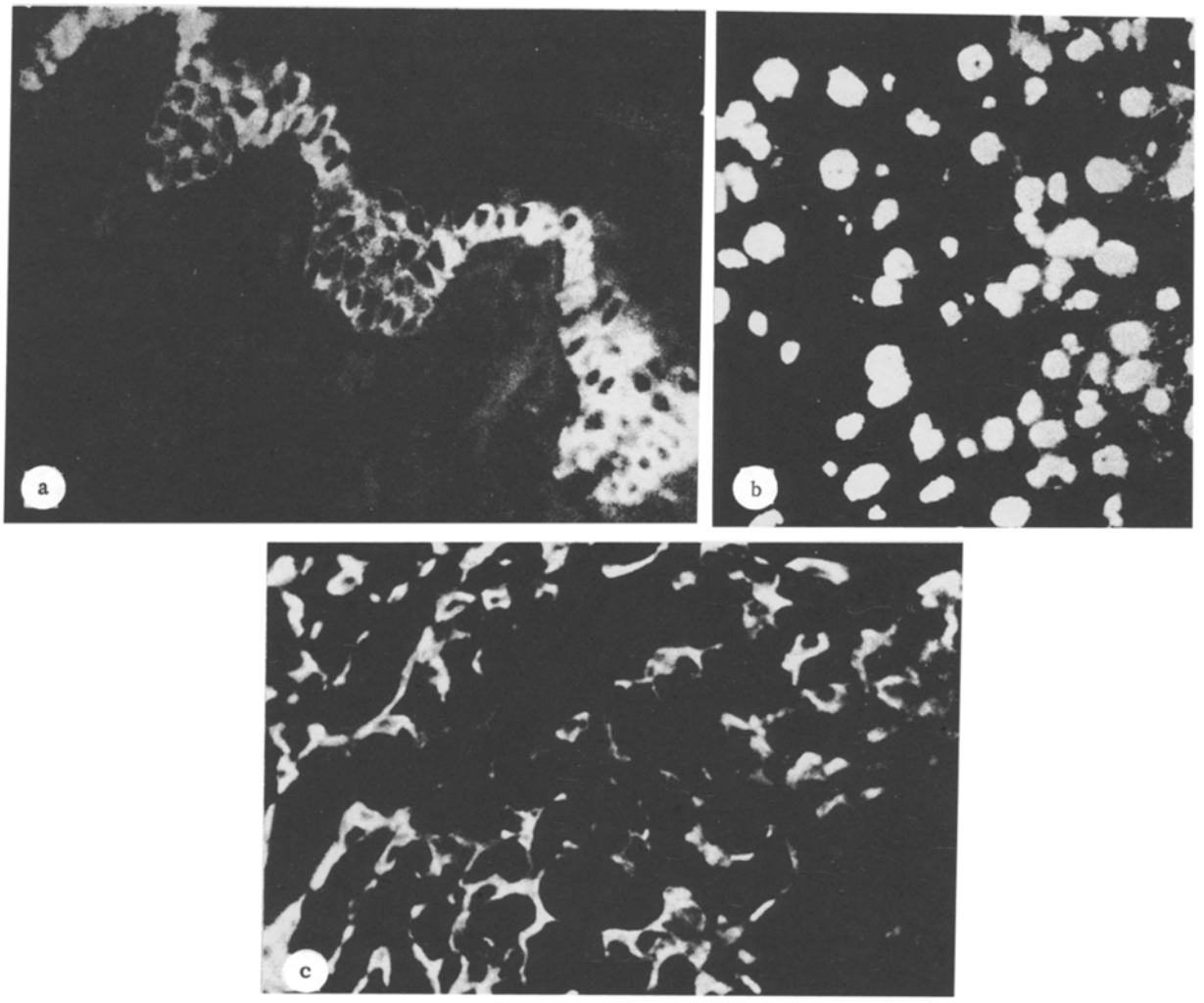

Fig. 1. Testing CR from mice immunized with A-PSC, conjugated with PEL, on unfixed frozen tissue sections. a) Reaction with stratum basale of epithelium on section of human skin; b) reaction with nuclei on section through liver tissue of BALB/c mouse; c) reaction with epithelial cells of human thymus. Sera diluted 1:32. IIFT. Objective 40, homal 3.

immunization with group A streptococci treated with pepsin, antibodies to A-PSC were detected in a titer of 1:800-1:1600. The sera of four animals reacted with the stratum basale of the skin epithelium. Some sera also reacted with layers of the epithelium above the stratum basale. No antibodies to cell nuclei were found. The sera of animals immunized with conjugates Nos. 1 and 2 also were tested on sections through the human and mouse thymus. Under these circumstances the reaction with the epithelium of the cortex and medulla of the thymus was observed only when sera obtained by immunization with conjugate No. 2, and reacting with the basal epithelium of the skin, were tested (Fig. 1c). It was thus shown that during immunization with A-PSC, conjugated with synthetic PEL, an immune response could be obtained to A-PSC in BALB/c mice.

As a result of immunization with conjugate No. 2, in which the content of A-PSC was 2.4 times higher than that of conjugate No. 1, higher antibody titers to A-PSC were obtained. Under these circumstances, after the first cycle of immunization antibodies reacting with epithelium of the skin and thymus were already found. Inhibition of these reactions with A-PSC confirmed that these antibodies were aimed at a determinant common to both A-PSC and epithelium. It was shown that these antibodies are autoantibodies, for reactions with epithelium were discovered in a syngeneic system (on tissues of BALB/c mice). The discovery of autoantibodies to cell nuclei in high titers in sera obtained after prolonged immunization with conjugate No. 2 is interesting. The appearance of these autoantibodies has evidently nothing to do with the presence of a corresponding CR-antigenic determinant in A-PSC, for A-PSC does not inhibit

these reactions. It must be pointed out that antibodies to epithelial tissues and also to cell nuclei are not found in animals after injection only of PEL or of A-PSC with Freund's adjuvant. The reason for the absence of antibodies to nuclei during immunization with pepsin-treated streptococcus calls for further study. In the present investigation only a small number of sera reacting with thymus epithelium, and obtained a short time after the beginning of immunization, were studied.

We know [10] that the epithelium of the thymus constitutes a microenvironment in which maturation and differentiation of T lymphocytes take place. The earlier appearance of autoantibodies to the epithelium of the thymus during immunization with A-PSC, conjugated with PEL, was possibly the cause of development of the immunoregulatory disturbances leading to the appearance of autoantibodies to nuclei. The problem of the damaging action of autoantibodies to the CR-determinant of A-PSC on the epithelium of the thymus and also the causes of appearance of autoantibodies to cell nuclei require further study. Animals immunized with conjugates of A-PSC with PEL can be used as an experimental model with which to study these problems.

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MONOCLONAL ANTIBODIES TO ANTIGENS ON HUMAN NEUTROPHILS, ACTIVATED T LYMPHOCYTES, AND ACUTE LEUKEMIA BLAST CELLS

G Yu. Miterev, G. F. Burova, M. S. Puzhitskaya, S. V. Danilevich, and T. I. Bulycheva

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Monoclonal antibodies (MCA) to differential antigens of human hematopoietic and lymphopoietic cells are currently widely used for marking leukocyte cell populations under normal conditions and in various pathological states, primarily in malignant diseases of the hematopoietic system [5, 14]. The use of these MCA provides extensive opportunities for the development of fundamentally new methods of diagnosis [5] and treatment [13] of hemoblastoses, and this, in turn, indicates the necessity of obtaining MCA to a broad spectrum of differential antigens of hematopoietic cells and a thorough study of the cellular and molecular specificity of the antigenic determinants revealed by them.

Group for Immunotherapy and Immunology of Leukemias, Central Research Institute of Hematology and Blood Transfusion, Moscow.

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