SUPPRESSION OF CYTOTOXIC CELLULAR REACTIONS DURING ANTIBODY PRODUCTION TO RHAMNOSE DETERMINANTS OF GROUP A STREPTOCOCCAL POLYSACCHARIDE, CROSS-REACTING WITH SKIN EPITHELIAL ANTIGENS

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The high level of antibodies to group A streptococcal polysaccharide (A-PS) in active rheumatic fever [4] evidently plays a role in the development of the autoimmune process. Determinants (DT), cross-reacting (CR), with various epithelial cells of the thymus and skin have been found in A-PS with the aid of poly- and monoclonal antibodies [6, 11].

The epithelium of the thymus has been shown to be a microenvironment in which various T-lymphocyte subpopulations mature [10]. Autoantibodies to epithelial antigen in the basal layer of the skin and the cortical and medullary zones of the thymus, CR with A-PS, have been found in active rheumatic fever [7]. Focal lysis and destruction of the epithelial cells of the thymus, containing epidermal antigens, also have been found, and their cytoplasm has been shown to contain bound immunoglobulins and complement [9]. It has been suggested that autoantibodies to DT of A-PS, CR with these structures of the thymus (the so-called endocrine epithelium), lead to immunoregulatory disturbances (IMRD), characteristic of autoimmune processes. Due to the heterogeneity of the thymus epithelium it is possible that other IMRD also may develop [5, 6].

One way of proving these hypotheses may be an experimental study of IMRD arising under the influence of antibodies to various CR DT of A-PS.

The aim of the investigation was to determine the effect of autoantibodies to skin epithelium, CR with various DT of A-PS, on cellular cytotoxic (CT) reactions connected with delayed-type hypersensitivity (DTH) to microbial antigens. The CT test to lymph node (LN) macrophages was used in an autologous system [1, 12]. DTH was produced to BCG antigens.

EXPERIMENTAL METHOD

Strains of group A streptococci (No. 6/49), L (43/50) (Prague collection) and A-variant (from M. McCarty, USA) were used. The V-PS of the A-variant of streptococcus contains only rhamnose determinants and does not contain group-specific (GS) DT of A-PS, including β-N-acetylglucosamine [8]. Heat-killed streptococci were treated with pepsin (A-PTS, L-PTS, V-PTS). A purified preparation of A-PS was obtained from A-PTS [8]. HCl-extracts containing cell wall proteins of group A streptococcus and purified M-protein were prepared [13]. Tuberculin (Leningrad Institute of Vaccines and Sera) was used. BALB/c mice (45 mice weighing 18-20 g) were immunized with A-PTS [2]. Sera were obtained 1 day before immunization (control), 1 week after the end of immunization (EIM), and 4 weeks later (end of the rest period – ERP). After blood had been taken in EIM or ERP, and also from 20 nonimmunized animals, 60 μg of BCG was injected as a single dose intraperitoneally, in Freund's incomplete adjuvant (Calbiochem, USA). Antibodies to various DT of A-PS were determined by enzyme immunoassay (EIA): to GS DT on a culture of A-PTS, and to rhamnose DT common to different groups of streptococci, and to an L-PTS culture during inhibition of V-PTS [2]. The L-PTS was chosen because the DT common to A-PS and L-PS is not CR with antigens of epithelial cells [6].

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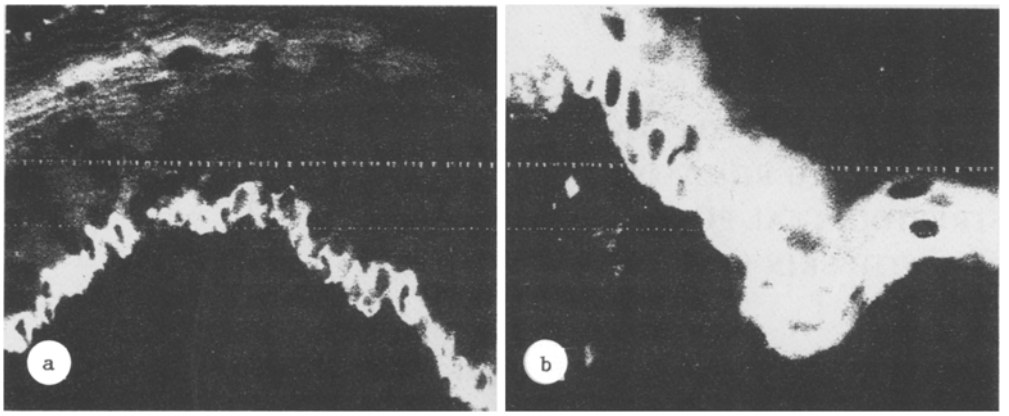

Fig. 1. Reaction of sera with cells of different layers of skin epithelium of BALB/c mice on immunization with pepsin-treated culture of group A streptococcus. a) Luminescence of cytoplasm of cells in basal layer, b) luminescence of cytoplasm of cells in basal and superbasal layers of epithelium.

Autoantibodies to antigens of skin epithelial cells were studied by the indirect immunofluorescence method (IIFM) [11] on sections of skin from the lip of BALB/c mice with antibodies to mouse IgG, labeled with fluorescein. The Lyumam I-1 fluorescent microscope with 40× objective was used. A homal 3× objective was used for photography. The reaction of antibodies to GS DT of A-PS with epithelium was abolished by A-PTS or A-PS. To inhibit the reaction of antibodies to rhamnose DT with epithelium, V-PTS was used [2]. The CT test was used during DTH in an autologous system [1, 12] in the modification in [3]. A suspension of mesenteric LN cells (17 million cells/ml), containing effector lymphocytes and macrophage target cells, 0.2 ml was added to wells of a 96-well panel (Flow Laboratories, England). In the experiments the cells were cultured with tuberculin (25 μg/ml), and in the control without antigen, or with M-protein (25 μg/ml), or with the HCl-extract (50 μg protein/ml). After culture for 18-20 h at 37°C and after removal of the nonadherent cells, macrophages stained with neutral red were counted [3] under an inverted microscope (Leitz). The cytotoxic index (CI) was determined in [3]. The results were subjected to statistical analysis by the usual method.

EXPERIMENTAL RESULTS

When the mouse sera were tested by the IIFM at EIM, autoantibodies to various cytoplasmic antigens of the skin epithelial cells were found in 94 ± 8.6% of cases (titer 1:16-1:64; Fig. 1). In 64 ± 11.5% of cases the autoantibodies reacted only with cells of the basal layer of the epidermis, and when tested by EIA, they contained a high level of antibodies to GS DT of A-PS (titer of antibodies to A-PTS ≥ 12,800). Antibodies to rhamnose DT were not found. In 36 ± 11.5% of cases sera containing antibodies to GS DT of A-PS (titer 1600-6400). In 61 ± 10.2% of sera, reactions were found (titer 1:16-1:32) simultaneously with cells of the basal and superbasal layers of the epithelium (Fig. 2) simultaneously. These sera contain, besides antibodies to GS DT of A-PS, antibodies whose titer was higher than that in EIM, to rhamnose DT of A-PS (titer 3200-6400). The reaction with epithelial cells at ERP, just as at EIM, was inhibited by A-PS and by a culture of A-PTS. Unlike at EIM, at ERP the V-PTS culture abolished the reaction not only with cells of the superbasal layer, but also with cells of the basal layer of the epidermis. In the unimmunized mice weak reactions (titer 1:8) with cells of all layers of the epithelium were observed in only 9 ± 5.5% of cases.

As a result of determination of DTH in mice sensitized with BCG, positive CT reactions were found only when tuberculin was used, but not with M-protein or with HCl-extract. In the group of mice which were unimmunized but sensitized with BCG, the mean value of CI in the presence of tuberculin was 58 ± 3.3%. In animals immunized with A-PTS and sensitized with BCG, the mean value of CI at EIM was 48 ± 6.4% (Fig. 3). In animals sensitized with BCG at ERP and not containing antibodies to rhamnose and to GS DT of A-PS, which are CR with epithelium, before sensitization the values of CI (45 ± 5.8%) likewise did not differ from CI in the control group. Conversely, in mice containing antibodies to rhamnose DT of A-PS, which are CR with epithelium, at ERP, virtually no CT reactions were found after sensitization with BCG. The mean value of CI was 1 ± 0.58%. Thus, marked suppression of DTH to BCG antigens was found in the animals of this group in the presence of autoantibodies reacting not only with the superbasal, but also with the basal layer of the epithelium. Both these reactions were linked

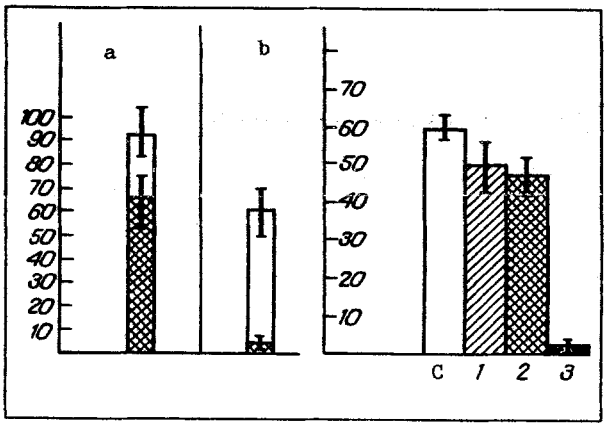

Fig. 2 Fig. 3

Fig. 2. Frequency of discovery of antibodies to various DT of A-PS, cross-reacting with antigens of cells of basal and superbasal layers of skin epithelium, at different times of immunization with streptococcus. Abscissa, time of immunization; ordinate, percentage of animals; shaded column — reaction with cells of basal alyer; unshaded — with cells of basal and superbasal epithelium; a) EIM, b) ERP.

Fig. 3. CT-effect during DTH to BCG antigens in mice containing antibodies to various DT of A-PS, CR with epithelial antigens. Abscissa, group of animals; ordinate, CI (in per cent). C) Control group of mice, not immunized with streptococcus. 1) EIM, sera contained high titers of antibodies to GS, DT, CR with cells of the basal layer of epithelium; 2) ERP, sera did not contain antibodies to CR determinants of A-PS and do not react with epithelium; 3) ERP, sera contained antibodies to rhamnose DT of A-PS, reacting with cells of basal and superbasal layers of epithelium.

with antibodies to rhamnose DT of A-PS, for they were abolished not only by the A-PTS culture, but also by V-PTS. The suppression effect correlated (r = 0.95) with the presence of antibodies to rhamnose DT of A-PS. At EIM, autoantibodies reacting with antigens of cells of the basal and superbasal layers of epithelium also were found in one-third of the animals. Autoantibodies to the basal layer at EIM were directed not to rhamnose DT, as at ERP, but toward GS DT of A-PS, and they were found in high titers. Suppression of the CT reactions during DTH was not found in this group. Thus, autoantibodies to different epithelial antigens, CR with A-PS can evidently differ in their action on cellular CT reactions. Inhibition of the CT reactions was observed or was absent depending on the presence of antibodies against different CR DT of A-PS, and therefore was not connected with suppression of DTH by large doses of streptococcus. Inhibition of the CT-effect also is observed in CBA mice in the presence of antibodies to rhamnose DT of A-PS [3]. Addition of lymphoid cells obtained from mice immunized with A-PTS, in which this inhibition effect is clearly defined, to LN cells of animals with DTH to BCG antigens, led to suppression of the CT-reactions. Antibodies to rhamnose DT of A-PS in vitro had no direct action of lymphocytes of the control animals with DTH and did not lead to abolition of the CT-reactions [3].

The results may be explained on the grounds of the hypothesis [5] that autoantibodies to CR DT of A-PS can induce IIFM because of their action on the epithelial cells of the thymus, which contain common antigens with the epidermis. Autoantibodies to epithelium, CR with rhamnose DT of A-PS, and which also induce suppression of cellular CT-reactions, may perhaps prevent the onset of autoimmune processes in streptococcal infections. Conversely, autoantibodies to basal epithelium, which are CR with GS DT of A-PS, may perhaps disturb the suppressor effect. Whether these autoantibodies cause damage to the endocrine epithelium of the thymus, which ought to facilitate the development of an autoimmune process [5], and what effect autoantibodies inducing suppression of cellular reactions may have on the epithelium of the thymus, only further study will show.

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CELLULAR MECHANISMS OF SUPPRESSION OF T LYMPHOCYTE PROLIFERATION BY LUNG CELLS IN EXPERIMENTAL TUBERCULOSIS

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The course of chronic infections diseases caused by intracellular pathogens, including *Mycobacterium tuberculosis*, in laboratory animals and man is accompanied by the development of suppression of the immune response [1, 11]. It is also known that immunocompetent lung cells in healthy animals have a suppressor action on certain immunologic reactions [7, 8]. However, the effects of cells of the lung, i.e., the organ of specific localization of tuberculus infection, on the immune response in tuberculosis have not been studied, and this is particularly true of the relations between specific and nonspecific immunosuppression in this infection.

Previously [2], the writers described a high level of suppressor activity of cells isolated from the interstitial tissue of the lung of mice infected with the virulent strain *M. tuberculosis* H37Rv. Suppression took place through the action of cells adherent to plastic and to nylon wadding, and characterized, besides by nonspecific activity, by a marked antigen-specific component, because small doses of suppressor cells inhibited only proliferation of T lymphocytes immune to mycobacterial antigens, in response to stimulation by tuberculin (PPD). High doses of lung cells suppressed the response to other antigens also, suggesting the existence of at least two suppressor mechanisms in foci of tuberculosis infection. In this paper we described the results of a study of the cellular mechanisms of immunosuppression in mice with tuberculosis.

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