A GROUP A STREPTOCOCCAL POLYSACCHARIDE STIMULATING NONSPECIFIC CYTOTOXIC REACTIONS IN AN AUTOLOGOUS SPLEEN CELL SYSTEM

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Group A streptococcal polysaccharides (A-PSC) as a common determinant with one of the epidermal antigens represented in the thymus [9]. Subsequent investigations using monoclonal antibodies revealed two determinants in the A-PSC molecule which cross-react with epidermal antigens of the epithelial tissue of the thymus [4, 5]. The antigenic similarity of the A-PSC molecule with epithelial factors of the thymus and also the presence of receptors for A-PSC on some lymphocytes in that organ [3] suggest that A-PSC, like epithelial factors of the thymus, possesses immunomodulating activity, which can be manifested at the level not only of thymus lymphocytes, but also of peripheral lymphoid organs. The aim of the present investigation was to test this hypothesis.

During the formation of delayed-type hypersensitivity (DTH) to microbial antigens, cytotoxic lymphocytes capable of causing lysis of target cells in the presence of a specific antigen (an inducer of DTH), appear in the lymphoid organs [12, 13]. Later investigations showed that autologous adherent cells (macrophages) from lymph nodes (LN) and spleen can be used as target cells [6, 10]. On the basis of these data a cytotoxic test was developed on autologous LN and splenic adherent cells (AC), which because of its high sensitivity and specificity can be used to determine DTH and to study the role of individual antigens in the development of this phenomenon [1, 2].

The aim of this investigation was to study the effect of A-PSC on the development of cytotoxic reactions in an autologous system of LN and spleen cells in mice with DTH (sensitization with BCG) and in normal animals.

EXPERIMENTAL METHOD

BALB/c and CBA mice aged 16-18 weeks received an intraperitoneal injection of BCG (60-90 μg/mouse). The presence of DTH to BCG antigens was determined in vivo as edema of one limb 24 hours after injection of tuberculin (25-30 μg protein in 0.04 ml of physiological saline) into it [1, 2].

In the control, physiological saline alone was injected into the limb.

A parallel cytotoxic test was set up on autologous AC from mesenteric LN and spleen, as described previously [1, 2]. The cells of these organs were suspended in Eagle's medium containing a 10% solution of fetal calf serum. After lysis of the erythrocytes, to enrich the spleen cell suspension with lymphocytes, some of the AC were first removed from it by incubation of the original suspension at 37°C for 45 min in cultural flasks. Nonadherent cells were collected, their concentration adjusted to 2×10^7 cells/ml, and transferred in a volume of 0.9 ml to flat-bottomed tubes (1 × 2.5 cm). A-PSC and (or) tuberculin and other antigens were added in a volume of 0.1 ml to the experimental tubes. The final concentration of A-PSC in the tubes was 30 μg, of tuberculin and M protein 25 μg, HCl extract of group A streptococcus 20 μg, and bovine serum albumin (BSA) 50 μg to 1 ml of medium. Medium alone (0.1 ml) without antigen and A-PSC was added to the control tubes. The results were read after 18 h on LN AC and after 36 h on splenic AC. The AC were stained beforehand with neutral red or trypan blue. The cytotoxic index (CI) was calculated by the formula:

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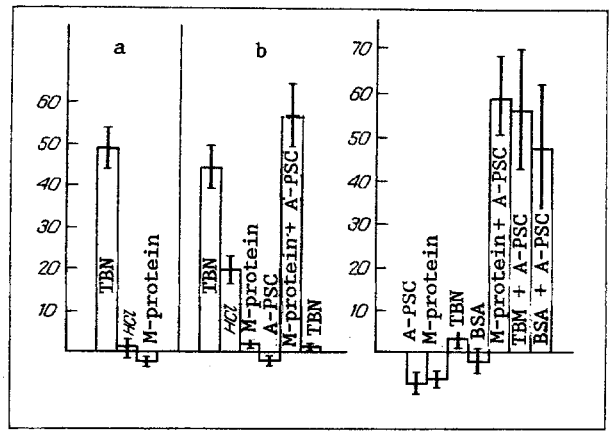

Fig. 1 Fig. 2

Fig. 1. Cytotoxic effect in autologous system of LN and spleen cells during DTH to BCG antigens. a) cytotoxic effect in autologous system of LN cells; b) cytotoxic effect to autologous system of spleen cells. Here and in Fig. 2: TBN) tuberculin; A-PSC) group A streptococcal polysaccharide; M-protein) type-specific cell-wall protein of group A streptococcus; HCl) HCl-extract of group A streptococcal antigens; BSA) bovine serum albumin. Abscissa, CI) in per cent.

Fig. 2. Stimulating effect of A-PSC on nonspecific cytotoxic reactions in autologous system of normal mouse spleen cells. Legend as to Fig. 1.

$$CI = \frac{A-B}{A} \cdot 100\ \%,$$

where A is the average number of AC per field of vision in the control, and B the same in the experiment.

The HCl extract was obtained from culture of group A streptococcus, type I or 29 by the traditional Lancefield's method. A-PSC was isolated by the foramide method from a culture of group A streptococcus, type I, treated with pepsin [7]. The method of isolation of the M-protein of group A streptococcus was described in [11], where a commercial preparation of tuberculin (Kazan, USSR) and of BSA (Moscow, USSR) was used.

EXPERIMENTAL RESULTS

DTH to tuberculin was found in the BALB/c and CBA mice on the 6th-8th day after sensitization by BCG, in the form of edema of the limbs. In parallel tests in an autologous system, a clearly defined cytotoxic effect was observed on LN AC in the presence of tuberculin (Fig. 1a). The mean value of CI was 49 ± 5.0%. In the control, on addition of nonspecific antigens contained in the HCl-extract of group A streptococcus to the medium, no cytotoxic effect was observed. By contrast, in an autologous system a cytotoxic effect on splenic AC of these mice was observed not in the presence of tuberculin (CI 44 ± 5.2%), but also on addition of the HCl-extract of group A streptococcus (20 ± 3.3%) or two of its components simultaneously – M-protein and A-PSC, to the medium (CI 37 ± 4.2%). The presence of A-PSC or M-protein alone in the culture medium did not lead to the development of a cytotoxic effect (Fig. 1b).

These observations are evidence that the spleen, unlike LN, contains cells capable of exerting a cytotoxic action on autologous AC under the influence not only of the specific antigen (tuberculin), but also of the nonspecific (M-protein). For a nonspecific cytotoxic effect to be exhibited under these circumstances, the simultaneous presence of nonspecific antigen and of A-PSC in the medium was necessary.

In view of these results, in the next series of experiments the effect of A-PSC was studied on development of the nonspecific cytotoxic effect in normal animals. For this purpose spleen cells from normal BALB/c or CBA mice were cultured in the presence of A-PSC and one of the antigens: tuberculin, M-protein, or BSA. It will be clear from Fig. 2 that addition of A-PSC

to the medium simultaneously with any of these antigens led to the development of a distinct cytotoxic effect. The mean value of CI on addition of (A-PSC + tuberculin), (A-PSC + M-protein), or (A-PSC + BSA) to the medium was 56 ± 5.4, 58 ± 5.2, and 47 ± 9.6%, respectively. In the control, when normal mouse spleen cells were cultured in the presence of antigens or of A-PSC only, death of AC was not observed.

The results are thus evidence that the nonspecific cytotoxic effect observed in an autologous system on splenic AC under the influence of the HCl-extract of group A streptococcus in animals sensitized by BCG is not connected with the development of DTH. It is evident that cells which, under the influence of A-PSC, can stimulate a cytotoxic action, are present in the spleens of animals sensitized with BCG and also of normal animals, unlike in LN. The action of A-PSC alone is not sufficient to activate cytotoxic cells, and an additional antigenic stimulus is essential. In our experiments antigens acted as such a stimulus: tuberculin, group A streptococcal M-protein, and BSA. It is also interesting that a nonspecific cytotoxic effect arises independently of the development of DTH and is observed in normal animals.

As has already been stated, two determinants common with epidermal antigen (or antigens) of the thymic epithelium are present in A-PSC [4, 5, 9]. Antibodies to these determinants of the A-PSC molecule are autoantibodies [4, 5]. It has been suggested that the action of these autoantibodies may lead to damage to the thymic epithelium and, as a result of that, to a disturbance of differentiation of thymic lymphocytes [4, 5, 9].

Meanwhile, the results of this investigation demonstrate that A-PSC can exert a direct influence on certain lymphocyte subpopulations of the peripheral lymphoid organs and, in particular, of the spleen. The influence of A-PSC may be connected with its action on effector cells or on regulatory subpopulations of T-cells; in both cases, moreover, receptors for A-PSC must be present on the cells.

As already pointed out, the thymus contains a subpopulation of lymphocytes with receptors for A-PSC [3]. The presence of such receptors on spleen cells is confirmed by the results of the present investigation. Data according to which nonspecific suppressor T cells as a receptor for rhamnose, one of the main components of the A-PSC molecule [8], are interesting in this connection. A cross determinant including rhamnose in its composition, common with one of the epidermal antigens of the thymus, has been found with the aid of monoclonal antibodies in A-PSC [4, 5]. It can be postulated on the basis of existing data that the immunomodulating effect of A-PSC is due to the fact that through the intermediary of its crossed determinant it interacts with a receptor on lymphocytes intended for the corresponding epithelial antigen (factor) of the thymus and stimulates its function.

Thus, A-PSC, as a carrier of the two determinants, can, first, facilitate damage to the thymic epithelium by autoantibodies directed against their common epitopes; second, it can play the role of functional analog of the thymic factor, and exerts a direct action on T-lymphocyte subpopulations and, perhaps, on other elements of the immune system also.

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