EFFECT OF SOME BACTERIAL ANTIGENS ON IMMUNE RECEPTORS OF MOUSE LYMPHOCYTES

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The action of various antigens of bacterial nature on lymphocytes is now generally familiar. Attention has been paid in some publications to the study of the effect of bacterial products on surface receptors of lymphocyte membranes. Proof has been obtained of the existence of receptors for lipopolysaccharides (LPS receptors) on the membrane of B lymphocytes [4-6, 8].

The aim of this investigation was to study the effect of the complete LPS antigen of Shigella sonnei and exotoxins of some clostridia (Cl. tetrani, Cl. botulinum) on immune receptors of lymphocytes. The effect of these agents was studied by the direct immunofluorescence and immune rosette formation reactions.

EXPERIMENTAL METHOD

Altogether 66 experiments were carried out on 370 BALB/c mice weighing 18-22 g. A cell suspension was prepared from the spleens of the mice by gentle homogenization. All manipulations with cells and also dilution of the test products were carried out in medium 199. Lymphocytes isolated by Böyum's method [3] were diluted so that each 0.2 ml of suspension contained 4 million cells.

Direct Immunofluorescence Test. A suspension of lymphocytes was poured in a volume of 0.2 ml into each of a series of centrifuge tubes which contained different dilutions of the bacterial products for testing in the same volumes: complete LPS antigen obtained from microorganisms of the Shigella sonnei group by Westphal's method [9] at the I. I. Mechnikov Research Institute of Vaccines and Seria; toxin of Cl. tetani (1 mouse MLD, 0.0001 mg); toxin of Cl. botulinum (1 mouse MLD, 0.001 mg) from the N. F. Gamaleya Institute of Epidemiology and Microbiology. The preparations were diluted when required. Lymphocytes were tested for viability and for the toxic effect on exposure to the products used with the aid of 0.1% trypan blue solution. The percentage of damaged lymphocytes was determined immediately after their isolation from the spleens of the mice (initial determination), and also after counting of the fluorescent cells in the control and experimental samples. This took place 7-12 h after isolation of the cells. The time of exposure of the lymphocytes to different dilutions of the test products, the temperature conditions used, repeated centrifugation, and so on, were taken into account. Meanwhile two controls were set up – K_1 and K_2.

K_1) The sample of lymphocyte suspension in 0.2 ml of medium 199 without addition of the test products was kept in the refrigerator throughout the experiment. After addition of the luminescent serum from the 3rd washing, the initial percentage of luminescence of the lymphoctes was determined and their viability verified (7-12 h after isolation).

K_2) The lymphocyte suspension in this control was subjected to the same tests as the experimental samples except treatment with the test products. In this case also the percentage of luminescent lymphocytes was determined and their viability was verified at the same times as in control K_1.

All the experimental tubes and those of K_2 were incubated at 37°C for 30 min. The tubes were constantly shaken. After washing twice in the cold, all tubes including K_1 and

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K_2 were treated with 0.2 ml of luminescent rabbit serum against albino mouse globulins in a working dilution (N. F. Gamaleya Institute of Epidemiology and Microbiology). Before the experiment the serum was adsorbed for 1 h at room temperature on an acetone liver powder of BALB/c mice. All the samples were then put in a refrigerator for 30 min, after which the lymphocytes were washed 3 times in the cold.

The cells were examined under the "Lyuman I-3" luminescent microscope. The number of luminescent cells among 200 cells was counted. The number of luminescent lymphocytes was compared before and after their treatment with the test products. Full details of the techniques were described earlier [2].

Immune Rosette Formation Test. In this part of the research 18 experiments were conducted on 122 mice. The animals were immunized with a 10% suspension of sheep's red blood cells in a volume of 0.2 ml intravenously. The mice were killed on the 6th day and cell suspensions prepared from their spleens, as stated above, in medium 199 with a final cell concentration of 10^7/ml. The viability of the cells was determined by the method described above. Rosette-forming cells (RFC) were detected by Zaalberg's method [10]. Two controls (K_1 and K_2) were set up as indicated above. The RFC were counted in a whole Goryaev's chamber in both grids. The mean result was taken. A type $NfpK_2$ binocular light microscope (VEB Carl Zeiss, Jena) was used. The number of RFC was expressed per 10^6 lymphocytes.

EXPERIMENTAL RESULTS

Exposure to complete LPS antigen, obtained from microorganisms of the Shigella sonnei group, led to a decrease in the number of luminescent lymphocytes. This decrease depended on the concentration of the preparation used. High concentrations induced more active inhibition of luminescence of the cells (Fig. 1). For instance, whereas in the control (K_2) the percentage of luminescent lymphocytes was 13.25 ± 0.94 (and in the initial control K_1 it was 17.25 ± 1.38), when the concentration of the preparation was 6.4×10^{-3}, the number of luminescent cells was reduced by 4.7%, to 12.5% with a concentration of 3.2×10^{-3}, and so on, to 60.1% when the concentration of LPS was 0.1×10^{-3}. Inhibition of luminescence of the lymphocytes was significant compared with the control, starting with the use of the preparation in a concentration of 0.8×10^{-3}. Lower concentrations (1.6×10^{-3}-6.4×10^{-3}) inhibited luminescence of the cells by a lesser degree and not significantly. However, in these concentrations also, a tendency could be clearly noted for the number of luminescent cells to decrease.

The percentage of viable lymphocytes 7-12 h after their isolation (the time during which the experiment was performed) varied from 89 to 75% depending on the concentration of the preparation used. The initial percentage of viable cells (immediately after isolation) was 97.6%, in K_2 7-12 h after isolation of the cells it was 89.3%, and in K_1, after the same time interval, it was 89.7% (Fig. 1). This suggests that the effect of inhibition of luminescence of the lymphocytes is connected with the toxic action of the test LPS.

Treatment of the lymphocytes with Cl. tetani toxin (TT) in doses of 0.01 and 250 MLD (except the dose of 1 MLD) lowered the percentage of luminescent cells compared with the control by 2.4-15.8%. These differences were not significant (Fig. 2). All comparisons were made with K_2, in which the percentage of luminescent cells was 17.50 ± 0.80 (20.79 ± 0.40 in K_1). With a dose of 1 MLD of the preparation inhibition of luminescence was maximal (28.4%), thus differing significantly from the control. With larger doses of the preparation (10-250 MLD) inhibition of luminescence was maximal (28.4%), thus differing significantly from the control. With larger doses of the preparation (10-250 MLD) inhibition of luminescence was reduced. Thus within the concentration range from 0.01 to 250 MLD the dose of 1 MLD was found to be critical, that at which inhibition of luminescence of the lymphocytes was greatest.

High concentrations of TT (500 MLD or higher) irreversibly and significantly inhibited luminescence of the cells. In a dose of 500 MLD, for instance, luminescence of the lymphocytes was reduced by 34.7%, with a dose of 1000 MLD by 41.0%, and with a dose of 2000 MLD, by 54.3%.

The percentage of viable cells under these circumstances changes as follows: 97.5% immediately after isolation of the cells, 85.3% in K_2 and 89.4% in K_1 7-12 h after isolation. After treatment of the lymphocytes with the toxin it fluctuated from 90.8 to 75.0% (Fig. 2).

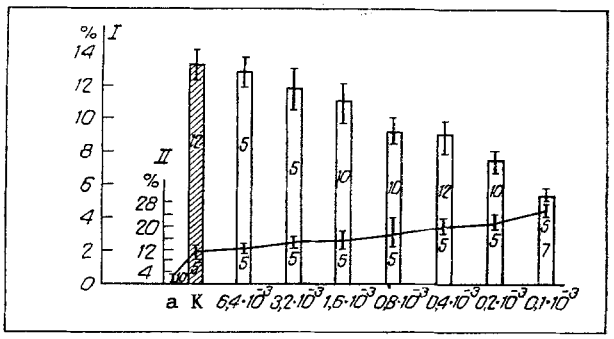

Fig. 1. Effect of complete LPS antigen of Shigella sonnei on luminescence of lymphocytes. Abscissa, dilution of preparation; ordinate: scale 1) percentage of luminescent lymphocytes; scale 2) percentage of damaged cells in test with 0.1% trypan blue solution. Columns show mean values (M ± m) of luminescent cells with different doses of LPS; curve plotted from data for mean values (M ± m) of percentage of damaged cells 7-12 h after their isolation and exposure to different LPS concentrations. a) Initial (immediately after isolation) percentage of injured cells, K) control (K_2). Numbers in columns and along curve denote number of experiments.

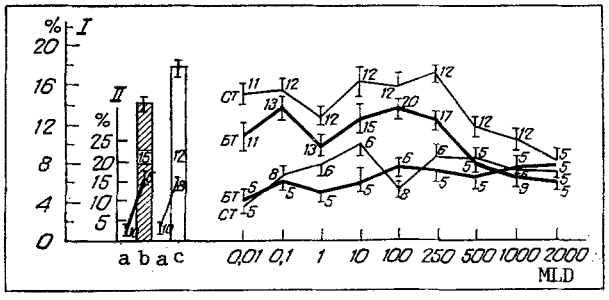

Fig. 2. Luminescence of lymphocytes after treatment with various doses of TT and BT. Abscissa, doses of preparations (in MLD); ordinate: scale 1) percentage of luminescent cells, scale 2) percentage of damaged cells in test with 0.1% trypan blue solution. Columns indicate mean (M ± m) values of luminescent cells in controls: b) control for series of experiments with botulinus exotoxin; c) with tetanus exotoxin. Curves in top part of figure show changes in luminescence of lymphocytes (mean values M ± m); in lower part – percentage of damage to cells. Thin curves – exposure to TT; bold curves – exposure to BT. Numbers in columns and along course of curves show number of experiments. a) Initial (immediately after isolation) percentage of damaged lymphocytes.

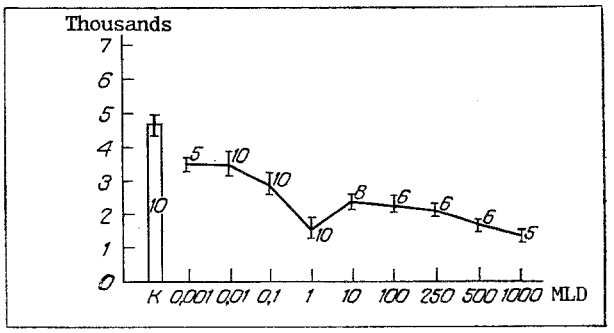

Fig. 3. Action of BT on immune rosette formation in mice. Abscissa, dose of prepatation (in MLD); ordinate, number of RFC (in thousands/10^6 lymphocytes); K) control (K_2); numbers indicate number of experiments.

Exposure of the lymphocytes from mouse spleen to another bacterial product, namely botulinus exotoxin (BT) caused inhibition of luminescence of the cells in accordance with the same general rule as for TT (Fig. 2). For instance, the percentage of luminescence of cells in the control (K_2) was 13.9 $\pm$ 0.67 (K_1 = 14.20 $\pm$ 1.04). Under the influence of the preparation in doses of between 0.01 and 250 MLD, luminescence of the cells was inhibited by 2.2-23.2% compared with K_2. However, the strongest inhibition (by 32.1%), just as in the case with TT, was observed during exposure to the preparation in a dose of 1 MLD.

Higher concentrations of the preparations (500, 1000, and 2000 MLD) caused inhibition of luminescence of the cells by 42.4, 54.8, and 56.8%. The percentage of viable cells in this series of experiments immediately after their isolation was 98, in K_2 7-12 h after isolation it was 84.1, and in K_1 after the same interval of time it was 89.6. After treatment of the lymphocytes with BT the percentage of viable cells 7-12 h after their isolation varied from 91.1 to 80.5% (Fig. 2).

The results showing the effect of BT on immune receptors of the lymphocytes, revealed by the direct immunofluorescence method, were confined by the method of immune rosette formation.

Treatment of mouse splenic lymphocytes with BT in doses of 0.001-1000 MLD caused a significant decrease (from 22.5 to 69.9% respectively) in the number of cells forming rosettes with sheep's red blood cells (SRBC; Fig. 3). All comparisons were made with K_2. In the control the number of RFC per 10^6 lymphocytes averaged 4640 $\pm$ 280. In K_1 it amounted to 6550 $\pm$ 510. The initial (immediately after isolation) percentage of viable cells was 97.4, in K_2 28-30 h after their isolation (the time in which the experiment was performed) it was 81.7, and in K_1 it was 84.3. After treatment of the lymphocytes with the toxin and after all manipulations with them had been done (see: Experimental Method), the percentage of viable cells 28-30 h after their isolation varied from 78 to 60.

The bacterial products which we tested thus inhibited luminescence of the lymphocytes and had an inhibitory action on immune rosette formation. The unique enhancement of the inhibitory effect of the bacterial toxins in a dose of 1 MLD was to some degree reversible, for a subsequent increase in the concentration of these products in the medium surrounding the lymphocytes was accompanied by facilitation of their inhibitory action. This effect may perhaps be due to action of expression of receptors from the cytoplasm of the lymphocytes to their surface.

The agents tested were able to disturb interaction of immune receptors of lymphocyte proteins of the sIgM and sIgD type, and giving interaction with Fc-receptors [7], with antibodies against mouse globulins, and also with SRBC. The ability of the bacterial products tested to block immune receptors of lymphocytes is unconnected with their toxic action, for the percentage of viable lymphocytes was reduced under the influence of the highest concentrations of these preparations by not more than 22% (varying from 3.6 to 21.7%) compared with the control (K_2). Blockade of the immune receptors by bacterial products is not a specific property of the agents studied, for it also takes place under the influence of certain choline-containing preparations, including acetylcholine, benzoylcholine, and phosphatidylcholine, of lectins, including PHA, ConA, LSG and, evidently, other agents [1, 2].

The problem of the molecular mechanisms of interaction between the bacterial agents tested in this study and immune receptors of lymphocytes requires further investigation.

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ROLE OF THE THYMUS IN REGULATION OF STROMAL CELLS TRANSFERRING THE HEMATOPOIESIS-INDUCING MICROENVIRONMENT IN STRESS

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The thymus is a universal organ which takes part in the formation of several complex homeostatic reactions of the individual [1, 4]. The writers showed previously on a model of immobilization stress that the thymus exerts its regulatory influence on hematopoiesis through the T-cell system [6]. In the modern view, processes of proliferation and differentiation of committed cells (precursors of myelopoiesis) are largely determined by the functional state of the stromal cells involved in the formation of the hematopoiesis-inducing microenvironment (HIM) of the bone marrow [5, 7]. The thymus can evidently influence the activity of the cells composing HIM [5]. However, information on this problem is extremely insufficient, and the role of the thymus in the regulation of HIM during exposure of the individual to extremal factors not possessing a myeloinhibitory effect has not been studied. Accordingly, the aim of the present investigation was to determine the role of the thymus in the regulation of the functional state of cells responsible for the transfer of HIM and the precursor cells of myelopoiesis during stress.

EXPERIMENTAL METHOD

Experiments were carried out on 850 male (CBA × C57BL)F_1 mice weighing 18-21 g (from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR). The animals were immobilized for 10 h in the supine position. At different times after immobilization the mice were killed by destruction of the spinal cord in the neck. The total number of myelokaryocytes (TNC) per femur was determined. The myelogram was counted in bone marrow films. The thymus of some of the mice was removed 1 month before immobilization or a corresponding mock operation was performed. The number of colony-forming units (CFU) was counted by the use of

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