CYTOTOXIC ACTION OF IMMUNE LYMPHOCYTES ON "ADHERENT" CELLS (MACROPHAGES) OF LYMPH NODES IN AN AUTOLOGOUS SYSTEM IN HYPERSENSITIVITY OF DELAYED TYPE

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The cytotoxic action of immune lymphocytes on "adherent" cells (macrophages; AC) of lymph glands from guinea pigs with hypersensitivity of delayed type (HDT) to streptococcal antigens and to tuberculin proteins was studied in an autologous system. Death of a considerable number of AC during cultivation of a suspension of lymph gland cells from animals sensitized with a streptococcal culture or Bacillus Calmette-Guerin vaccine (BCG) was demonstrated in the presence only of the specific antigen (thermostable fraction of streptococcus or of tuberculin respectively). The discovery of death of AC in an autologous system can be used as a specific and sensitive test with which to study HDT.

KEY WORDS: hypersensitivity of delayed type; immune lymphocytes; "adherent" cells, cytotoxic action.

In the modern view immune lymphocytes participate in injuries to tissues that are observed in hypersensitivity of delayed type (HDT) to microbial antigens and in autoimmune and other immunopathological processes. Meanwhile the role of macrophages, which are usually found in the zone of development of reactions of delayed type, in this process is not clear. If peritoneal exudate cells obtained from animals with HDT are cultured in the presence of specific antigen, the permeability of the lysosomal membranes is increased and some of the macrophages die [3-5]. On the basis of this observation it has been postulated that the liberation of enzymes from macrophagal lysosomes may be the cause of death of surrounding tissue cells. The writers showed previously [6] that lymph node cells obtained from guinea pigs with HDT against streptococcal or tuberculin antigens, in the presence of the specific antigen, caused death of a considerable number of cells of a monolayer culture of peritoneal macrophages. These investigations were carried out in an allogeneic system.

Suspensions of lymph gland cells obtained by the usual methods, without special separation, are known to contain a certain number of "adherent" cells (AC). These cells, in their morphology, their ability to adhere firmly to a surface, and their staining properties with neutral red behave as macrophages.

The object of this investigation was to study the cytotoxic action of immune lymphocytes in an autologous system on AC (macrophages) from lymph glands of animals with HDT.

EXPERIMENTAL METHOD

Noninbred guinea pigs were sensitized by injection of a culture of group A streptococcus, type 10 (strain Dochez NY-5) in Freund's incomplete adjuvant (10^{10} bacterial cells) or Freund's complete adjuvant

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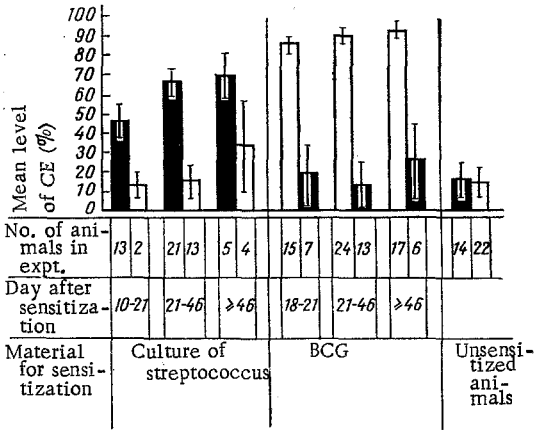

Fig. 1. Mean level of cytotoxic effect obtained on death of "adherent" cells (macrophages) of lymph nodes of animals with HDT, taken at different times after sensitization. Black columns) cultivation of suspension of lymph node cells with TST fractions; unshaded columns) with tuberculin.

(600 μg of BCG) into the foot pads. The thermostable fraction of streptococcus (TST fraction), prepared by Ando's method [7] in Verzhikovskii's modification [2], and tuberculin (Leningrad Institute of Vaccines and Sera) were used as the antigens for the intradermal tests and the cytotoxic text. The animals on which intradermal tests were performed to confirm the presence of HDT were not used in experiments to study the cytotoxic action.

The source of lymphocytes and macrophages was a suspension of cells from the inguinal, popliteal, femoral, and subclavian lymph glands of animals sensitized with a culture of streptococcus or BCG, or from unsensitized animals. Lymph gland cells obtained by expression from each animal separately were washed in a large volume of culture medium, and the number of living lymphocytes was counted after vital staining. The suspension was diluted to a concentration of $18 \cdot 10^6$–$20 \cdot 10^6$ living lymphocytes to 1 ml culture medium (medium No. 199 with 20% bovine serum, and penicillin and streptomycin, 50 units of each to 1 ml). Antigens prepared in the same medium were added at the rate of 100μg to 1 ml TST fraction or 25 μg to 1 ml tuberculin (calculated as protein).

After cultivation for 24 h at 37°C the supernatant with unattached cells was removed and replaced with a fresh portion of culture medium (without addition of antigen), and the incubation was continued. The number of living cells adherent to the glass, morphologically similar to macrophages and staining intensively with neutral red, was counted on the fifth to sixth day after vital staining. Fibroblast-like cells were not counted. Cells were counted in three or four tubes, in 15 fields of vision for each tube. The cytotoxic effect (CE) was determined by the equation:

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

where A is the mean number of cells in the control tubes, in which lymph node cells were cultivated without antigen; B is the mean number of cells in the experimental tubes, in which the same cells were grown initially for 24 h with antigen, and later in culture medium.

EXPERIMENTAL RESULTS

Intradermal tests showed that on the 22nd day and later after sensitization with streptococcal culture the animals developed a reaction of delayed type. This reaction attained its greatest intensity 24 h after intradermal injection of 5 μg TST fraction and it was characterized by severe induration and erythema (diameter 20-30 mm). Tests on the tenth day after sensitization showed no reaction in the skin, or only a slight erythema. In response to injection of tuberculin on the 18th day or later after sensitization with BCG, as a rule the reactions of delayed type were intensive.

After cultivation of the lymph gland cells of sensitized animals for 24 h in the presence of specific antigen, the formation of conglomerates of 5-15 AC surrounded by a dense circle of lymphocytes was observed; later the AC increased considerably in size, the vacuoles in them became more numerous, and after 72-96 h the number of AC diminished. These phenomena were more marked when lymph gland cells of animals sensitized with BCG were cultivated with tuberculin. Counts showed that the number of AC in the suspension of lymph gland cells obtained from different animals varied between 10 and 150 per field of vision; in the earlier stages after sensitization (until the 25th day), especially after sensitization with a streptococcal culture, the number of AC as a rule exceeded 30 per field of vision.

During incubation of lymph gland cells from animals sensitized with the streptococcal culture with the TST fraction (the cells were obtained on the 21st day and later) in 22 experiments out of 26 fewer than half of the AC were preserved compared with the number of cells in the control tubes. In 19 cases from this number of experiments the CE was over 60%. A lower mortality among AC was found when cells were taken from lymph glands obtained in the earlier stages (on the 10th and 18th-20th days) after sensitization with the streptococcal culture, when the intradermal reactions were either absent or still weak. In this group, which consisted of 13 animals, CE did not exceed 60%, but in 8 of them CE was 50% or less. Incubation with tuberculin of lymph gland cells from animals sensitized with BCG led to death of more than 80% of the AC. This high CE was observed when cells were used from lymph glands taken after the 18th day after sensitization (in the earlier stages after sensitization with BCG the animals were not tested). The cytotoxic effect continued at a high level for 2 months (period of observation).

To study the specificity of mortality of AC in the autologous system, lymph gland cells from animals sensitized with the streptococcal culture of BCG were cultivated with nonspecific antigen – with tuberculin or the TST fraction, respectively. In this case the AC as a rule were preserved on the glass in large numbers, and in most experiments CE was below 30% (Fig. 1). Similar results were obtained on cultivation of a suspension of lymph gland cells from unsensitized animals with the TST fraction or tuberculin, and in most cases CE was below 30%.

Comparison of the mean level of CE in the experimental series (incubation of lymph gland cells from animals sensitized with streptococcal culture or BCG, with specific antigen) and in the control (incubation of the same cells with nonspecific antigen or of cells of unsensitized animals with the given antigens), showed that the differences were significant. Significance of the differences was estimated by the formula:

$$\overline{X}_1 - \overline{X}_2 > tpS \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

In an autologous system, death of AC (macrophages) from the lymph glands of animals sensitized with streptococcal culture or BCG was thus established. A high CE (60-100%) was found during cultivation of a suspension of lymph gland cells from animals with a marked HDT but only with the specific antigens to which the animals were sensitized. Under these circumstances CE on AC was stronger for animals sensitized with BCG than with the streptococcal culture. Similar results were obtained previously in an allogeneic system when the cytotoxic action of a suspension of lymph gland cells obtained from animals with HDT to streptococcal antigens and to tuberculin proteins were studied during the action of peritoneal macrophages taken as target cells on the monolayer culture [6]. Death of macrophages observed in vitro in the autologous system evidently takes place also in vivo when sensitized lymphocytes come into contact with antigen. The "activation" and subsequent death of the macrophages must evidently lead to liberation of cytoplasmic and lysosomal enzymes. Enzymes of macrophages can exert a harmful action on the cells of the surrounding tissues and, in the first place, on the cells of tissues least resistant to the particular enzymes. These phenomena can evidently be the cause of development of immunopathological processes, especially in diseases of streptococcal etiology.

Determination of the percentage mortality of AC in an autologous system can be used as a specific and sensitive test for the presence of HDT.

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