CYTOTOXIC ACTION OF LYMPHOTOXIN ON A CULTURE OF MACROPHAGES

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The results of a study of the cytotoxic action of lymphotoxins on a monolayer culture of peritoneal macrophages, used as target cells, are described. Lymphotoxins were obtained after cultivation of a suspension of lymph gland cells from guinea pigs with hypersensitivity of delayed type to tuberculoproteins in the presence of the specific antigen (tuberculin) for 18 h. The lymphotoxins in 70% of experiments caused death of more than half of the macrophages of the monolayer, but only if counted on the 5th-6th day of cultivation. Death of the macrophages at later stages of cultivation than L-cells is evidently an indication of differences in the resistance of these target cells to the cytotoxic action of lymphotoxin.

KEY WORDS: hypersensitivity of delayed type; lymphocytes; lymphotoxin; cytotoxic effect; macrophages – target cells.

One of the manifestations of hypersensitivity of delayed type (HDT) in vitro is a cytotoxic action of the immune lymphocytes on target cells. The process taking place under these circumstances can be divided, in the modern view, into two phases. During the first phase, which is strictly specific, interaction takes place between immune lymphocytes and the corresponding antigen, with a consequent liberation of a factor from the lymphocytes known as lymphotoxin, with a cytotoxic action on target cells. During the second phase destruction of target cells of varied origin takes place under the influence of this factor [9]. The second phase is thus not specific. The study of the second phase of cytotoxic action is interesting because target cells of different origin may evidently differ in their sensitivity to the cytotoxic factor.

The results of a study of the cytotoxic action of lymph gland cells of guinea pigs with HDT to streptococcal antigens or tuberculin on a monolayer culture of peritoneal exudate cells taken as target cells were described previously [4]. Death of the macrophages began to be found after 72 h and it was particularly noticeable after incubation for 96 h with specific antigen. Meanwhile death of fibroblasts (L-cells) was usually observed after incubation for 48-72 h and it took place not only as a result of direct contact with the immune lymphocytes, but also through the action of lymphotoxin obtained after interaction between immune lymphocytes and specific antigen [6, 7, 10, 11].

The object of this investigation was to study the cytotoxic action of lymphotoxins obtained after cultivation of a suspension of lymph gland cells from animals sensitized with BCG, in the presence of tuberculin, on a monolayer culture of peritoneal macrophages taken as the target cells.

EXPERIMENTAL METHOD

Guinea pigs were sensitized by an injection of Freund's complete adjuvant into the plantar pad (600 μg of a killed culture of BCG per guinea pig). To prepare the lymphotoxins cells were obtained from the subclavian, axillary, popliteal, and inguinal lymph glands of guinea pigs with HDT or of unsensitized (normal) animals. The suspension of lymph gland cells was washed twice in a large volume of medium No. 199 (150 ml) and the number of living lymphocytes was counted after vital staining. The cell suspension was divided

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into two parts and again centrifuged. The resulting cell residue was resuspended to a concentration of $30 \cdot 10^6$ living lymphocytes to 1 ml in medium No. 199 with the addition of bovine serum (20%), inactivated by heating to 56°C, or in a solution of tuberculin (23-46 g/ml) made up in the same medium. (In these experiments the tuberculin was obtained from the Leningrad Institute of Vaccines and Sera.) The cell suspension thus obtained was poured into a tube or into tissue culture flasks. After incubation for 18-24 h at 37°C the supernatant was collected and centrifuged for 30 min at 1500 rpm until complete sedimentation of the cells, and 1 ml of the undiluted supernatant after decantation was added to each of a series of tubes containing a 1-2-day monolayer culture of peritoneal macrophages obtained from unsensitized guinea pigs. The cytotoxic action was determined by counting macrophages remaining attached to the glass on the 5th-6th day after addition of the experimental or control supernatants and incubation at 37°C. The experimental supernatant was obtained by cultivating a suspension of lymph gland cells from sensitized animals in the presence of antigen, the control supernatant by growing the cells in culture medium without addition of the antigen. Preparation of a monolayer culture of macrophages and of the suspension of lymph gland cells and the method of counting were described in detail in an earlier paper [4].

EXPERIMENTAL RESULTS

The cytotoxic action of supernatants prepared from lymph gland cells from 20 guinea pigs sensitized with BCG was studied. The macrophages were visibly enlarged 48 h and, in particular, 72 h after addition of the experimental supernatant and they contained more vacuoles and granules. On the 5th-6th day a marked decrease in the number of cells in the monolayer was found. After addition of this particular supernatant to the monolayer culture of macrophages in 14 cases less than half (20-50%) of the cells remained attached to the glass compared with the number of cells grown in culture in the presence of the control supernatant. Only in seven of 14 cases, however, did the cytotoxic effect reach 60-80%. On the addition of supernatants obtained after incubation of the lymph gland cells of unsensitized animals with antigen or without it to the monolayer culture of macrophages no cytotoxic action was observed. No appreciable differences were found when the number of cells was counted in tubes containing a monolayer culture of macrophages grown either in medium or in the presence of a working dose of tuberculin. However, in these tubes the number of cells of the monolayer was much smaller at the time of counting than in the tubes to which the control supernatants were added. The cells used to prepare the supernatants were from lymph glands obtained from animals at various times (13th-50th day) after sensitization with BCG. Tests of these supernatants showed no marked differences in their cytotoxic action.

The supernatants obtained after incubation of the lymph gland cells of animals with HDT in the presence of the specific antigen thus caused death of the cells of a monolayer culture of peritoneal macrophages. The cytotoxic effect was not always clearly defined. Only in one-third of cases did it reach 60-80%, whereas after addition of a suspension of intact lymph gland cells it did so in 84% of cases [4]. Nathan and coworkers [8] described the results of a study of certain functions of a monolayer culture of macrophages after growth with lymphotoxin for 72 h. They showed that during this incubation period the macrophages were activated and their phagocytic and adhesive properties were enhanced. However, these workers did not find death of the macrophages as a result of the action of lymphotoxin. As the results of the present experiments showed, the cytotoxic action both of the suspension of lymph gland cells and of the supernatant on macrophages from the peritoneal exudate was observed only if the cells were counted on the 4th and 6th days respectively. Meanwhile, according to other workers [6, 7, 10, 11], death of L-cells when cultivated with immune lymphocytes or with lymphotoxin was usually observed earlier (after 48-72 h) during incubation. Similar results were obtained by the writer in a study of the cytotoxic action of lymphotoxin prepared by the method described above on L-cells [5]. In view of these findings it can be postulated that the slight delay in death of the macrophages compared with death of the L-cells was due to differences in the resistance of these target cells to the cytotoxic action of lymphotoxin.

The mechanism of the cytotoxic action of immune lymphocytes has not yet been explained. The suggestion has been made that as a result of interaction between immune lymphocytes and specific antigen a factor causing activation of lysosomal enzymes in the target cells is liberated [11]. It has also been shown that during cultivation of peritoneal exudate cells obtained from animals with HDT in the presence of the specific antigen alone for 24 h the permeability of the lysosomal membranes of the macrophages becomes increased [1-3]. Addition of the supernatants, and also of lymph gland cells from animals with HDT, and of the specific antigen caused appreciable activation of the macrophages after 48-72 h. The possibility cannot be ruled out that lysosomal enzymes are liberated from the activated macrophages, as a result of the increased permeability of the membranes, into the surrounding medium and that this causes the death of

the target cells themselves. It may be expected that the same sort of phenomenon would develop in a focus of inflammation associated with HDT, where usually many monocytes are found. In the presence of specific antigen and of immune lymphocytes, activation and subsequent death of the macrophages, which are cells rich in enzymes, evidently take place. The liberation of enzymes into the surrounding medium may give rise to destruction of the cells and, in particular, cells of the most sensitive tissues.

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