# CYTOTOXIC ACTION OF LYMPHOCYTES ON PERITONEAL MACROPHAGES OF MICE WITH CONTACT DERMATITIS CAUSED BY DINITROCHLOROBENZENE

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Contact dermatitis was produced in BALB/c mice by percutaneous application of a solution of dinitrochlorobenzene (DNCB). On the 20th day of the experiment, when the skin test with DNCB was positive, peritoneal macrophages were taken from DNCB-sensitized and intact mice and cultured in flat-bottomed tubes for 48 h. A suspension of lymphocytes from peripheral lymph nodes was added to them. When incubated together with cells of DNCB-sensitized mice the lymphocytes had a cytotoxic action on the target cells (peritoneal macrophages), as shown by staining with Trypan Blue after 48 h. When cells from intact mice were incubated with lymphocytes, lysis of the macrophages was not observed.

KEY WORDS: *cytotoxic effect of lymphocytes; peritoneal macrophages; contact dermatitis; dinitrochlorobenzene.*

Contact sensitivity (CS), as regards its dynamics and manifestations, is one of the most typical forms of hypersensitivity of delayed type (HDT) [12]. Investigations of contact allergic dermatitis in man [4] and many experimental studies of CS caused by derivatives of dinitrophenol, picryl chloride, oxazolone, and other chemical compounds, have shown that a chronic inflammatory process in which proliferation based on an HDT reaction is predominant develops in CS [1]. Investigations of immunocompetent cells in CS has consisted largely of the study of the ability of lymphocytes from peripheral lymph nodes [10], bone marrow, and thymus [7] to form rosettes. As a rule, all the experiments were carried out on guinea pigs. The cytotoxic effect (CTE) of lymphocytes during interaction with target cells has been studied mainly in antitumor and transplantation immunity [11] and has also been demonstrated in diseases with an autoimmune mechanism. CTE associated with interaction of sensitized lymphocytes with antigen-containing target cells (peritoneal macrophages) has been established in experimental tuberculosis and after vaccination in mice [3].

The object of this investigation was to study the cytotoxic action of lymphocytes obtained from animals with contact allergic dermatitis on syngeneic target cells, specifically on macrophages from peritoneal exudate.

## EXPERIMENTAL METHOD

Experiments were carried out on 40 male BALB/c mice. Contact dermatitis was produced by application of a 2% or 25% solution of dinitrochlorobenzene (DNCB) in acetone to the freshly depilated skin of the lateral surface of the body. The 2% solution was applied daily and the 25% solution 3 times in the course of 2 weeks. Macrophages of the peritoneal exudate induced by injection of thioglycol broth and obtained from sensitized and intact animals were grown for 48 h in flat-bottomed Leighton's tubes. Each tube contained 20,000-250,000 macrophages in 1 ml of medium No. 199 with the addition of L-glutamine and antibiotics, 20% bovine serum, and 10% lactalbumin. Lymphocytes from sensitized and intact animals were then added to the tubes in the ratio of 80 viable lymphocytes to 1 macrophage in the experiments of series I and

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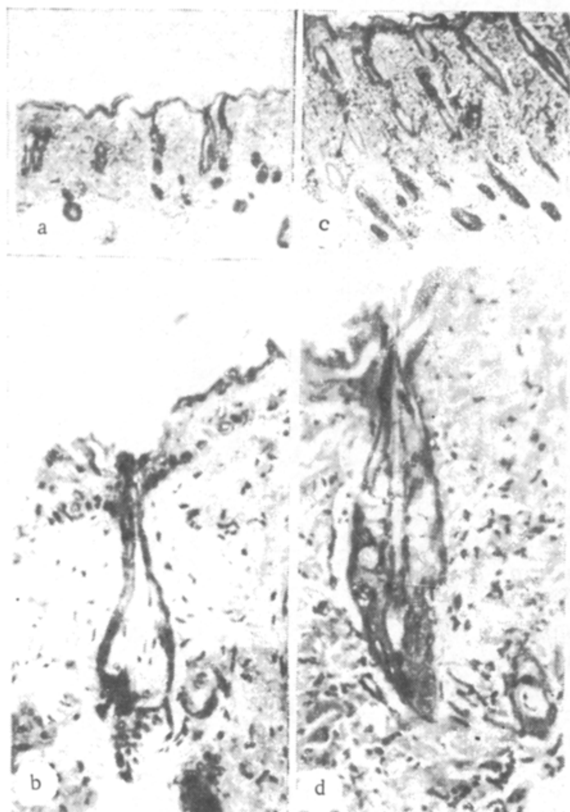

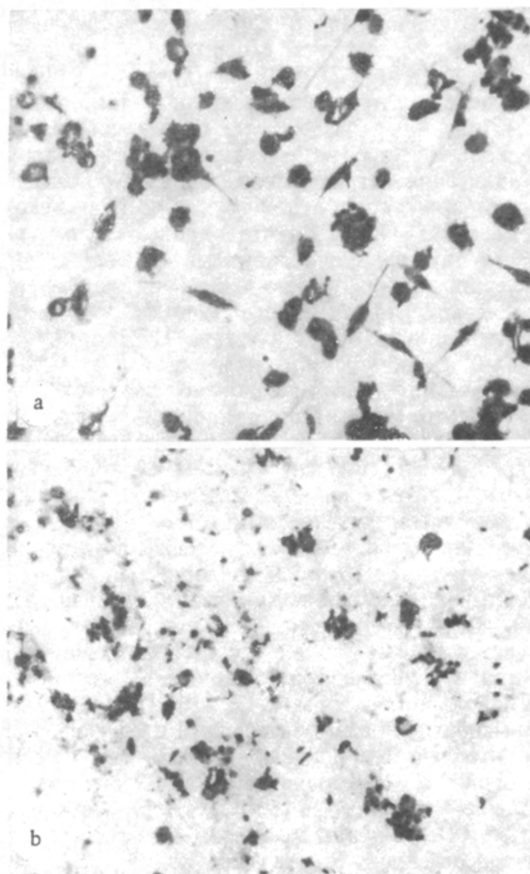

Fig. 1 Fig. 2

Fig. 1. Skin of mice 48 h after skin testing with DNCB: a) skin of intact mouse after application of acetone without DNCB (control, 40×); b) the same under high power (control, 150×); c) greatly thickened skin of a sensitized mouse in region of reacting application (skin test) of 0.02% solution of DNCB in acetone; marked monocytic infiltration and edema (experiment, 40×); d) the same under high power (experiment, 150×). Hematoxylin—eosin.

Fig. 2. Cytotoxic action of lymphocytes on peritoneal macrophages of mice with contact dermatitis caused by DNCB: a) combined incubation of macrophages and lymphocytes from intact mice (control); growth of macrophages after addition of lymphocytes; b) combined incubation of macrophages and lymphocytes from sensitized mice, marked lysis of macrophages after incubation for 48 h with lymphocytes. Azure—eosin, 200×.

40 lymphocytes to 1 macrophage in series II. Two series of experiments were carried out at an interval of 2 weeks, and 15 mice were used in each series; 10 intact mice acted as the control. Lymphocytes were expressed from the lymph nodes and their viability determined by staining with 0.1% Trypan Blue and 0.1% eosin. Lymphocytes were added to each tube containing macrophages in 1 ml of medium No. 199 with 10% lactalbumin. The reaction was set up in four different ways: 1) incubation of macrophages and lymphocytes of intact mice; 2) macrophages of intact mice with lymphocytes of sensitized mice; 3) macrophages of sensitized mice with lymphocytes of intact mice; and 4) macrophages and lymphocytes of sensitized mice. The course of the reaction was monitored after 6, 12, 24, and 48 h. The results (the number of living macrophages on staining with 0.1% Trypan Blue) were estimated after 48 h by the formula:

$$\frac{a-b}{a} \cdot 100\%,$$

where $a$ is the number of living macrophages (on average per field of vision under a magnification of 200×) when macrophages and lymphocytes were incubated in one of the first 3 variants of the test (control); b the number of living macrophages after incubation of macrophages

TABLE 1. Cytotoxic Action of Lymphocytes on Peritoneal Macrophages of Mice with Contact Sensitization to DNCB

| Series of experiments | Variant of reaction | Number of living macrophagess (M ± m) | Cytotoxic effect, % |
|---|---|---|---|
| I | Macrophages and lymphocytes of intact mice (control) | 35±2,05 | 31,4 |
| | Macrophages and lymphocytes of sensitized mice (experiments) | 24±5,11* | |
| | Macrophages of intact and lymphocytes of sensitized mice | 18±1,48 † | 43,3 |
| | Macrophages of sensitized and lymphocytes of intact mice | 11±1,52 † | |
| II | Macrophages and lymphocytes of intact mice (control) | 20±4,24 | |
| | Macrophages and lymphocytes of sensitized mice (experiments) | 20±4,05 | |

*$P > 0.05$.
†$P < 0.01$ for difference between experiment and control.

and lymphocytes from sensitized animals (experiment). As a result, the cytotoxic index was obtained in percent. The mean number of macrophages per field of vision was calculated for 5-6 tubes in each group. The significance of the difference between the means for the experimental groups was assessed with the aid of Student's criterion.

Some of the mice (from which cells were not taken for determination of the CTE) were subjected on the 20th day of the experiments to a skin test with application of a 0.02% solution of DNCB in acetone to the skin of the lateral surface opposite to the site of the sensitizing applications. An equal volume of acetone without DNCB was applied to the control mice. The intensity of the reaction was assessed by Chase's method [6] after 24 and 48 h. The animals were killed 48 h after the skin test. The skin in the region of dermatitis (the site of DNCB applications) and of the skin test, the lymph nodes, thymus, and spleen were investigated histologically.

## EXPERIMENTAL RESULTS

After a single application of the 25% solution or 3 or 4 applications of the 2% solution of DNCB, thickening and induration were found, followed by the development of necrosis of the epidermis and the formation of granulation tissue. In the region of the skin test 48 h after application of a 0.02% solution of DNCB, considerable thickening and induration of the skin were observed macroscopically. Histologically, compared with the control (Fig. 1) considerable thickening of the skin was found on account of marked infiltration of the dermis, the dermo-epidermal zone and around the appendages of the skin by monocytes and edema. In the regional lymph nodes (inguinal and axillary) the number of lymphocytes was increased, with the formation of follicular concentrations of lymphocytes with hyperbasophilic cytoplasm.

The results showed (Table 1) that lymphocytes from sensitized animals had a cytotoxic action on macrophages from the peritoneal exudate of syngeneic mice sensitized by the same antigen (DNCB). On combined incubation of macrophages of intact animals with sensitized or intact lymphocytes or of macrophages from sensitized mice with intact lymphocytes (the first 3 variants of the CTE test), good growth of macrophages was observed with well-marked spread, with predominance of cells giving off processes, and with the appearance of fibroblast-like

cells (Fig. 2a). Adhesion of the added lymphocytes to the macrophages was ill defined, and lysis only of individual cells was observed after combined incubation for 48 h.

After addition of lymphocytes of sensitized mice to macrophages of the same group (experiment), adhesion of the lymphocytes to the surface of the macrophages was found, with the formation of lymphocytic muffs. After combined incubation for 48 h most of the macrophages had undergone lysis (Fig. 2b). Many (about 80%) of the lymphocytes had also died as a result of these cellular interactions, as staining with Trypan Blue revealed.

Lymphocytes with a cytotoxic action on target cells (peritoneal macrophages) of sensitized mice were thus found in CS. The effect was evidently connected with the presence of the hapten (DNCB), bound with a carrier protein, in the peritoneal macrophages. This indicates that in CS immune lymphocytes with a lytic action on target cells bound with the antigen play an essential role in the development of immune inflammation taking place on the basis of HDT. In this case cytolysis, mediated by T lymphocytes in which an important place is occupied by specific binding of the effector T lymphocyte with the target cell [8], evidently occurs. The absence of a CTE on the addition of sensitized lymphocytes to intact macrophages probably indicates that the effector function of the lymphocytes is aimed actually at the hapten-containing complex and not at the intact syngeneic cells (macrophages), as might be expected during the development of the so-called autoimmune component of the process. However, the latter hypothesis requires special verification. It is evidently only during combined incubation of lymphocytes and macrophages of sensitized mice that the "receptors" of the lymphocytes exactly match the antigens of the target cells, an essential requirement for manifestation of the CTE [2]. It is interesting to note that on the addition of half the number of lymphocytes per target cell in the experiments of series II (40:1) the intensity of the CTE was significantly greater than in series I (80:1), in which the difference between the experiment and the control was not statistically significant. With a concentration of 40:1, the proportions of the cells were evidently more optimal for manifestation of the CTE, at least in this particular model. Tuberculin and contact HDT have been included in a special group of "allergic inflammation" [5] or immune reactions [9] different from the so-called cytotoxic reactions (graft rejection, certain autoimmune diseases). The results described above, and also those obtained previously by the study of CTE in experimental tuberculosis and after vaccination, helped to fill in details of the picture of the mechanisms of development of CS and tuberculin allergy.

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