Antigen detected by ILO-GM-1 MA was expressed on blast cells of three of the three patients with acute monoblastic leukemia (AMonL), on leukocytes of patients in the chronic phase of chronic myeloid leukemia (CML), in three of four patients with a blast crisis of CML (BC CML). In a patient with BC CML on whose blast cells the antigen was not expressed, a lymphoid variant of BC CML was diagnosed. Antigen detected by IKO-GM-1 MA was represented on blast cells of three of the eight patients with acute myeloid leukemia (AML) and two of four patients with AMML.

IKO-GM-1 antibodies did not react with blast cells of five patients with acute lymphoblastic leukemia (ALL), four patients with lymphosarcoma (LSA), and with the B lymphocytes of 10 patients with CLL (Table 2); they likewise did not react with blast cells from a patient with acute promyelocytic leukemia. The antigen was well represented on monocytes of a patient with chronic monocytic leukemia.

The characteristics described above indicate that antigen detected by IKO-GM-1 MA is presented on mature cells of the myeloid series and on monocytes at all stages of differentiation. This character of expression of the antigen on blast cells of patients with AML and AMML can be explained by heterogeneity of the pool of leukemic cells, in which both early precursors of the myeloid series and also more mature cell forms are represented [5].

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# ACTION OF HYDROCORTISONE ON CYTOTOXIC REACTIONS IN DELAYED-TYPE HYPERSENSITIVITY TO MICROBIAL ANTIGENS


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In delayed-type hypersensitivity (DTH) sensitized lymphocytes in the presence of specific antigens and also macrophages activated by lymphokines have a cytotoxic action on target cells and secrete soluble factors (lympho- and macrophagotoxins), with cytotoxic properties [2, 7, 8, 14]. It has been shown that secretion of macrophagotoxin, like that of lymphotoxin, takes place under the influence of specific antigens to which the animals are sensitized. It has been shown that macrophagotoxin differs from lymphotoxin in certain of its parameters [1]. The mechanisms of the cytotoxic action of lymphotocytes and of lympho- and macrophagotoxins on target cells evidently have certain special features and may differ in their sensitivity to the inhibitory action of steroid hormones. It has been shown that corticosteroids inhibit secretion of certain lymphokines by lymphocytes [4, 13] and also the action of lymphokines on macrophages [6]. Meanwhile the inhibitory action of hydro-


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cortisone (HC) on the production and cytotoxic activity of lympho- and macrophagotoxins has not been studied. It is likewise not yet clear whether steroid hormones have an inhibitory action on the cytotoxic activity of T cells in DTH. According to some data there is a decrease in the cytotoxic activity of T cells under the influence of dexamethasone in vitro [12]. At the same time it has been shown that precursors of T-killers, sensitized with H-2 antigens, are resistant to HC administered in vivo [5]. It is thus not yet clear which component of the cytotoxic effect in DTH to microbial antigens is most sensitive to the inhibitory action of corticosteroids.

The aim of this investigation was to study the effect of different doses of HC, injected into animals in vivo during DTH to BCG antigens, on the production of lympho- and macrophagotoxins in the presence of specific antigens, and also on the cytotoxic activity of sensitized lymphocytes.

## EXPERIMENTAL METHOD

Noninbred guinea pigs weighing 250-300 g were sensitized by a single injection of 600 μg of BCG into the footpads. Starting from the 20th or 28th day after sensitization, HC was injected intramuscularly into the animals daily for 7 days. The total doses of HC per animal were 30 or 60 mg. Microcrystalline HC (from Richter, Hungary) was used for the injections.

In each experiment cells were obtained from peripheral lymph nodes (PLN) and the peritoneal cavity of animals of four groups (normal animals; guinea pigs sensitized with BCG; without receiving HC and after injection of HC in a dose of 30 or 60 mg per animal), as described previously [1, 3]. A suspension of PLN cells containing $20 \cdot 10^6$ living lymphocytes was used for the cytotoxic test in an autologous system [8]. On the addition of specific antigens (tuberculin in a concentration of 25 μg/ml) the cytotoxic action of sensitized lymphocytes on adherent cells (AC), namely PLN macrophages, which in this case were used as target cells, was determined. After culture of the PLN cells for 18-20 h at 37°C in the presence of antigens (tuberculin) preparations containing lymphotoxin were obtained by decantation [1].

Cells from the peritoneal cavity were obtained without preliminary stimulation of the animals. Macrophages were separated from lymphocytes by making use of their ability to adhere to glass. To prepare supernatants containing macrophagotoxin, a monolayer culture of AC obtained from a suspension of cells from the peritoneal cavity, containing $2.5 \cdot 10^6$ macrophages (cells stained with neutral red), were cultured in the presence of tuberculin. The concentration of lympho- or macrophagotoxin in the supernatants was determined by the cytotoxic test on a culture of fibroblasts (L-cells) [1, 2].

The cytotoxic index (CI) in all tests used was determined by comparing mortality of the target cells in the experiment and control. Supernatants obtained by decanting cultures of lymphocytes or macrophages grown in the absence of tuberculin were used as the control, and in the autologous cytotoxic test the number of AC was determined in tubes to which no antigens were added. The CI was calculated by the formula:

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

where A denotes the mean number of target cells in the control and B the number of cells in the experiment. The results were subjected to statistical analysis by Student's t test.

## EXPERIMENTAL RESULTS

Injection of HC in a total dose of 30 mg into animals sensitized with BCG caused a decrease in cytotoxic activity of the supernatants obtained after culture of periotoneal macrophages in the presence of specific antigens. The mean value of CI when supernatants of macrophages were tested was reduced by about half (from $48 \pm 6$ to $23 \pm 3\%$) compared with the supernatants of sensitized animals, not receiving HC (Fig. 1). Meanwhile, after injection of this dose of hormone the cytotoxic activity of the supernatants obtained after culture of PLN cells from the same animals, in the presence of specific antigens, was virtually unchanged.

With an increase in the total dose of HC to 60 mg, simultaneously with a decrease in cytotoxic activity of the supernatants obtained by culture of macrophages, a decrease also was observed in the cytotoxic activity of supernatants of PLN cells. The mean value of CI in tests on supernatants of PLN cells after injection of 60 mg of HC fell from $66 \pm 5.3$ to $33 \pm 2.3\%$. Consequently, CI fell almost by half compared with CI in sensitized animals not receiving HC or receiving HC in a dose of 30 mg. An increase in the dose of the hormone in this case did not lead to a more marked decrease in cytotoxic activity of the supernatants of the peritoneal macrophages compared with the corresponding value obtained when a dose of 30 mg was given.

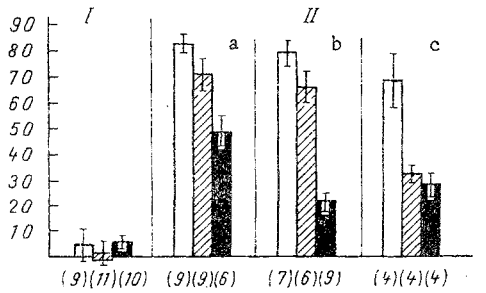


Fig. 1. Effect of different doses of HC on cytotoxic effect of lymphocytes and supernatants containing lympho- or macrophagotoxin, in animals with DTH to BCG antigens. Ordinate, mean value of CI (in %). I) Normal animals; II) animals sensitized with BCG: a) without injection of HC, b) receiving HC in a dose of 30 mg, c) HC in a dose of 60 mg. Unshaded columns – value of CI for death of PLN AC (macrophages) in an autologous system under the influence of sensitized lymphocytes; shaded columns – values of CI for death of fibroblasts (L-cells) under influence of lymphotoxin, black columns – the same, but under the influence of macrophagotoxin. Number of animals given in parentheses.

Differences in the mean values of CI in tests of supernatants of macrophages and PLN cells of animals sensitized with BCG, and treated with HC, and supernatants of cells from normal (unsensitized) animals were statistically significant ($P \leq 0.05$). Thus supernatants obtained by culture of peritoneal macrophages and PLN cells after injection of HC continued to have a cytotoxic action on L-cells, unlike supernatants from cells of normal animals. Meanwhile the level of their cytotoxic action was much lower than that of supernatants obtained by culture of cells from animals not receiving HC.

After injection of HC into sensitized animals a tendency was noted for the cytotoxic action of the lymphocytes on autologous macrophages in the presence of specific antigens to diminish, especially when the dose of hormones given was 60 mg (Fig. 1). However, differences in the mean values of CI for death of adherent PLN cells of sensitized animals receiving or not receiving HC are not statistically significant.

Some investigators have found that the action of corticosteroids leads to a decrease in permeability of the lysosomal membranes of macrophages and to inhibition of secretion of lysosomal enzymes [7, 9, 10, 11]. They showed that doses of steroid hormones almost 10 times greater than those inhibiting secretion of lysosomal enzymes by macrophages [15] are needed to inhibit the secretion of one of the lymphokines, namely macrophage migration inhibiting factor. Our own results are evidence that to inhibit secretion of lymphotoxin in DTH larger doses of HC also are required. Meanwhile, lower doses of HC inhibit secretion of macrophagotoxin, possibly due to reduced secretion of lysosomal enzymes by macrophages.

The results of the present experiments also show that administration of HC in vivo to animals with DTH to microbial antigens virtually does not depress the cytotoxic activity of sensitized lymphocytes. Similar results, as was mentioned above, were obtained in a study of the action of HC on precursors of T killer cells, sensitized to H-2 antigens [5].

It must also be pointed out that when the cytotoxic test was set up in DTH in an autologous system HC did not cause any decrease in sensitivity of the target cells to the cytotoxic action of lymphocytes. The basis for this view is absence of an inhibitory action of the hormone on the cytotoxic effect when using AC (PLN macrophages) as target cells in an autologous system.

The results are evidence of the need to look for other preparations more effective than HC for inhibiting the cytotoxic activity of T cells in DTH.

By the methods used in the present investigation it was possible to determine the action of HC on the cytotoxic activity of lymphocytes during DTH in an autologous system, and also on the process of secretion of lympho- and macrophagotoxins. These methods must be used in the future to test other pharmacological preparations and to study the mechanisms of their action on the cytotoxic effect in DTH.

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