EFFECT OF HYDROCORTISONE ON NEUTROPHIL FUNCTION

D. N. Mayanskii and O. P. Makarova

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Under the influence of glucocorticoids the total peripheral blood neutrophil count changes and granulocytopoiesis develops [1, 7]. However, the functional capacity of the polymorphonuclear leukocytes (PML) has not been adequately studied after administration of these hormones, although this is an important problem because the glucocorticoid-dependent fluctuations of resistance to infection are largely dependent on the properties of PML.

The aim of this investigation was to study functional activity of peripheral blood neutrophils in mice at various times after administration of hydrocortisone (HC).

EXPERIMENTAL METHOD

Experiments wre carried out on male (CBA × C57BL)F_1 mice weighing 18-20 g, into which HC ("Gedeon Richter," Hungary) was injected intraperitoneally in a dose of 125 mg/kg. The effect of HC was studied in the same animals 2, 12, and 24 h and 3, 7, and 14 days after the injection. The state of PML function was deduced from the results of the nitro-BT test before and after microbial stimulation of the cells in vitro. The nitro-BT test reflected the ability of PML to activate oxygen-dependent metabolism and generation of biooxidants with bactericidal activity [4]. Blood for analysis was taken from the retro-orbital sinus. The nitro-BT test was carried out before and after addition of killed S. marcescens vaccine to whole blood in a dose of 2×10^9 microbial cells/ml as a stimulator of neutrophils (the i-NBT test) [3]. The percentage of PML with deposition of diformazan was calculated. At the same time the total leukocyte count and leukocyte formula were determined. In experiments in vitro HC ("Merck," West Germany), dissolved in dimethyl sulfoxide, was added to the incubation medium for the nitro-BT test in a final concentration of 7×10^{-6}, 3.5×10^{-6}, and 7×10^{-5} M. Two portions of medium were prepared for each dose of hormone. After incubation for 10 min with HC at 37°C, 25 µl of a 0.2% solution of nitro-BT ("Lachema, Chemapol," Czechoslovakia) and 25 µl of 1/15 M phosphate buffer were added to one portion of medium, and instead of the buffer, 25 µl of vaccine (2×10^9 bacterial cells/ml) was added to the other portion, and incubation continued for a further 30 min. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Injection of HC was followed after 2 h by a marked leukopenia, which intensified until 24 h, persisted until the 3rd day, and then gradually disappeared toward the 14th day of the experiment (Fig. 1). The leukocyte formula at all times of the investigation was dominated by neutrophils, but nevertheless the absolute number of blood neutrophils fell sharply. During the first day after injection of HC an increase was observed in the percentage of active neutrophils in the nitro-BT test (Fig. 1). An increase in activity of the cells in the circulating pool was recorded after 2 h. Values of the i-NBT test were increased toward 24 h after injection of the hormone. The results of the nitro-BT and i-NBT tests were inversely proportional to the total leukocyte count ($r = -0.749$; $r = -0.556$; $p < 0.05$).

Addition of HC to the peripheral blood in the in vitro system caused no significant change to the results of the nitro-BT test. However, additional stimulation of the neutrophils

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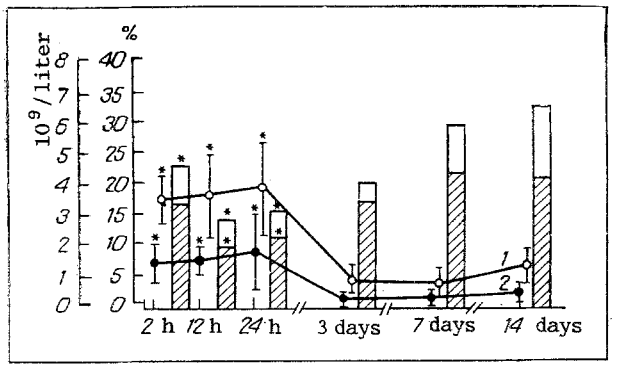

Fig. 1. Total leukocyte count, absolute neutrophil count, and values of nitro-BT and i-NBT tests in male (CBA × C57BL)F_1 mice after injection of hydrocortisone in a dose of 125 mg/kg. Unshaded columns – leukocytes, shaded – neutrophils (× 10^9/liter). 1) Nitro-BT test (in %); 2) i-NBT test (in %). *$p < 0.05$.

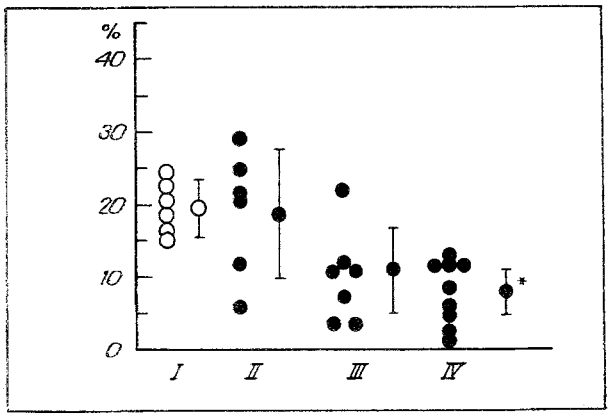

Fig. 2. Effect of hydrocortisone in vitro on results of i-NBT test (in %). I) Control; II) 7×10^{-6} M; III) 3.5×10^{-6} M; IV) 7×10^{-5} M. *$p < 0.05$.

by the vaccine after incubation of blood with the hormone revealed a dose-dependent inhibitory action of HC. As Fig. 2 shows, starting with a dose of 3.5×10^{-6} M, HC caused a decrease in the values of the i-NBT test. Dimethyl sulfoxide, in the concentrations used to dissolve HC, did not affect the results of the nitro-BT or i-NBT tests.

After injection of HC an increase in the percentage of active neutrophils, despite an absolute decrease in their number in the blood, was observed for 24 h. Activation of the pool can be regarded as a compensatory reaction to a sudden fall in the number of granulocytes. It has been shown that neutrophilic leukocytosis develops in response to acute stress and to moderate doses of glucocorticoids [1, 2]. Meanwhile, an absolute neutropenia is observed after administration of high doses of glucocorticoids [1]. The fall in the number of granulocytes is evidently associated with their more intensive sequestration in the lungs. For instance, in rats receiving HC in a dose of 1.25 mg/kg the number of neutrophils in bronchoalveolar washings from the lungs was increased by 60 times [5]. In our own experiments, HC-induced neutropenia was accompanied by increased sensitivity of blood neutrophils to microbial stimulation. Direct contact of peripheral blood with high HC concentrations in vitro led to a fall in the functional reserves of the neutrophil pool. This was manifested as lowering of the values obtained in the nitro-BT test after incubation of blood with the hormone in doses of 3.5×10^{-6} M and 7×10^{-5} M, whereas an HC concentration ten times less had no significant effect. The decrease in sensitivity of neutrophils to microbial stimulation after their treatment with high doses of HC in vitro could be due to inhibition of expression of C3- and Fc-receptors and inhibition of activation of complement along the classical and alternative pathways [6, 8].

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A DIFFERENTIATION FACTOR IS PRESENT IN BONE MARROW AND BLOOD SERUM OF NORMAL INDIVIDUALS AND PATIENTS WITH ACUTE LEUKEMIAS

O. A. Khoperskaya, O. V. Stolbovskaya, and T. A. Zershchikova

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Interest is increasing in the subject of factors activating cell differentiation both during early embryonic development and during differentiation of hematopoietic stem cells in the adult [2, 4, 5, 7]. Differentiation factors (DF) help to determine the fat of cells in the early stages of embryogenesis, but in adult animals they are essential compounds of the extracellular environment, which plays a leading role in the differentiation of stem and semistem cells [4, 5]. Whereas the role of DF in embryogenesis has been studied in fair detail, the role of DF in adult organisms has not yet been adequately investigated. Studies of the forced differentiation of malignant hematopoietic cells provide evidence of the influence of DF on these processes [1]. One of the most widely distributed DF is the factor which activates differentiation of mesodermal cell types: notochord, muscles, blood cells, mesenchyme, and mesothelium. It has been called mesodermalizing factor (MF) because of its ability to induce mesodermal derivatives in early development [10]. In the adult, it has been called systemic connective-tissue morphogen (SCTM), because of the direction of its action and its localization [4]. SCTM in adult animals is located in the extracellular matrices, bone, cartilage, blood serum [3, 8], and bone marrow [11, 12], and is synthesized by lymphocytes. It has been shown in experimental animals (rats, guinea pigs) that DF with mesodermalizing activity is not synthesized in the bone marrow in leukemia [11]. However, these observations have not been confirmed in man during the development of acute leukemias.

The aims of this investigation were: 1) to detect SCTM in bone marrow of healthy blood donors and to compare its concentration with that in the bone marrow of patients with acute

N. I. Vavilov Institute of General Genetics, Academy of Sciences of the USSR, Moscow.
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