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EFFECT OF THE DEGREE OF GLUCOCORTICOID SATURATION ON PERIPHERAL BLOOD CFUs LEVELS

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It was shown previously that large doses of glucocorticoids reduce migration of hematopoietic stem cells from the bone marrow into the spleen [5]. Moreover, on the basis of the results of a series of investigations into the effect of the pituitary-adrenal system on some aspects of function of CFUs it was concluded that this system has a regulatory role on migration and recirculation of hematopoietic stem cells [1, 2, 4] although the mechanism of action of different doses of glucocorticoids is not clear.

The aim of the present investigation was accordingly to study the action of dexamethasone, administered in different doses, on peripheral blood CFUs, depending on the concentrations of the hormone created by its administration.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL)F_1 mice. Dexamethasone phosphate (from Galenika, Yugoslavia) was injected intraperitoneally in doses of 0.005, 0.02, and 0.2 mg per mouse. Animals receiving physiological saline served as the control group. The peripheral blood CFUs level was determined 30 min and 1.5, 5, 24, and 48 h after injection of the hormone or physiological saline, by transplantation of 0.2 ml blood from the injected mice into lethally irradiated recipients. The number of CFUs in the peripheral blood of the donor mice was judged from the number of colonies growing on the spleen of recipient mice on the 8th day after transplantation [8]. The recipients were irradiated on the EGO-2 apparatus in a dose of 850 R with a dose rate of 120 R/min. The degree of saturation of the mice with dexamethasone following injection of the hormone was calculated on the basis of data given in [7]. The endogenous corticosterone level was determined 5 and 24 h after injection of dexamethasone by the method in [6]. The results were analyzed by Student's t test.

EXPERIMENTAL RESULTS

A tendency for the peripheral blood CFUs level to fall, although not significantly, was observed 30 min after injection of dexamethasone in a dose of 0.005 mg per mouse. The number of CFUs after 1.5 h was reduced to 58.1% ($P < 0.05$). After 5 h the number of circulating CFUs was back to normal, after 24 h their level was higher than in the control, and the initial values were reached 48 h after injection (Fig. 1).

A fourfold increase in the dose of dexamethasone (0.02 mg per mouse) was accompanied by a more prolonged fall in the number of circulating CFUs: After 30 min there was a very small decrease in the number of these cells, after 1.5 and 5 h the CFUs level was down to 51.7 and 48% respectively, after 24 h the number of CFUs in the peripheral blood was increased up to 169% of the initial values, and 48 h after injection the CFUs level remained considerably higher than initially, at 222% (Fig. 1).

Injection of dexamethasone in a larger dose (0.2 mg per mouse) gave rise to an intensive and prolonged decrease in the number of CFUs in the peripheral blood. After 30 min the fall in the blood CFUs level was very small, 1.5 h after injection their number had fallen to 25% of its initial value, the level remained low 5 and 24 h after injection of dexamethasone, but after 48 h the number of CFUs was back to the initial level (Fig. 1).

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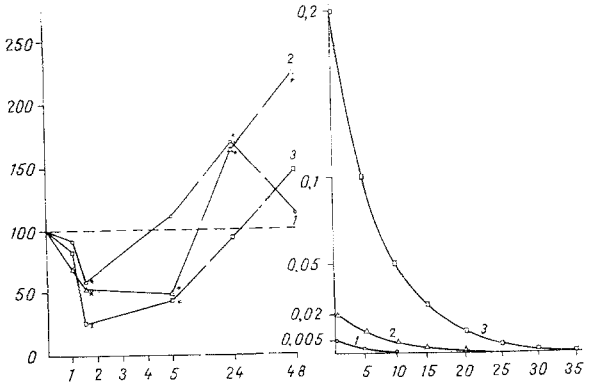

Fig. 1 Fig. 2

Fig. 1. Level of circulating CFUs after injection of dexamethasone in various doses: 1) 0.005 mg per mouse, 2) 0.02 mg per mouse, 3) 0.2 mg per mouse. Asterisk indicates that differences compared with control group (injection of physiological saline) are significant at $P < 0.05$. Abscissa, time (in h); ordinate, dose of hormone (in mg).

Fig. 2. Time course of dexamethasone excretion after administration in different doses. Abscissa, time (in h); ordinate, dose of hormone (in mg). 1, 2, 3) The same as in Fig. 1.

Dexamethasone, in the doses studied, thus caused phasic changes in the number of circulating CFUs: A fall in the number of stem cells in the peripheral blood was followed by a rise.

Plotting the time course of saturation of the mice with different doses of dexamethasone against circulating CFUs levels reveals a direct relationship between the degree of saturation and duration of circulation of the hormone in the animals and the decrease in the number of stem cells (Figs. 1 and 2). When dexamethasone was given in a dose of 0.2 mg per mouse high concentrations of the hormone in the body were created 1.5, 5, and 24 h after injection, and the number of CFUs in the peripheral blood was sharply reduced at these times. The higher the hormone concentration, moreover, the greater the fall in the number of circulating CFUs. By 30-35 h the hormone had been excreted and, as the experiments showed, after 48 h the peripheral blood CFUs level was back to normal. Investigation of the endogenous corticosteroid level showed that after 30 h it did not differ statistically significantly from the initial values (12.6 ± 3 μg% in the experiment and 8.8 ± 0.6 μg% in the control), evidence of normalization of the CFUs level when physiological concentrations of glucocorticoids were reached.

A smaller dose of dexamethasone led to an increase in its concentration 1.5 and 5 h after its injection only, and a fall in the number of circulating CFUs was observed at those times. The degree of hormone saturation after injection of this dose of dexamethasone was less than after a dose of 0.2 mg, and the fall in the number of CFUs in the blood also was less marked. The hormone had been eliminated from the body 20 h after injection. Determination of endogenous glucocorticoids at that time showed that their secretion by the adrenals of the mice had fallen to 47.8% of their initial level ($P < 0.05$). As Fig. 1 shows, the number of circulating CFUs was sharply increased at that time.

The hormone level was high 1.5 h after injection of 0.005 mg dexamethasone and the number of circulating CFUs was reduced. Practically all the dexamethasone had been excreted 5 h after its injection, hormone production and secretion of endogenous corticosteroids in the adrenals were blocked (secretion was down to 23.9% of the initial values; $P < 0.05$), and the level of circulating CFUs was raised.

The rapid effect of dexamethasone on the circulating CFUs pool can be linked with a redistributive mechanism by analogy with that for lymphocytes [3].

The results are further evidence of the regulatory role of glucocorticoids in the migration and recirculation of CFUs.

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EFFECT OF *Mycoplasma arthritidis* ON RECOVERY OF ERYTHROPOIESIS IN MICE AFTER ADMINISTRATION OF 5-FLUOROURACIL

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KEY WORDS: *Mycoplasma arthritidis*; 5-fluorouracil; erythropoiesis.

Experimental infection of mice with *Mycoplasma arthritidis* is accompanied by stimulation of endogenous colony formation in mice irradiated in a sublethal dose [1, 4] and contributes to the more rapid regeneration of erythropoiesis in plethoric mice [5] and also in mice which have previously been given repeated injections of small doses of actinomycin D [2]. The problem of the nature of the target cells for *M. arthritidis* and the mechanism of its action on hematopoietic cells remains unsolved.

It was therefore decided to study the action of *M. arthritidis* on recovery of erythropoiesis in mice in which hematopoiesis was disturbed as a result of a single injection of a sublethal dose of the cycle-specific cytostatic 5-fluorouracil (5-FU). It was postulated that if *M. arthritidis* affects the proliferative state of hematopoietic cells, its injection at different times relative to 5-FU ought to lead to significant changes in the time course of recovery of erythropoiesis after the action of the cytostatic.

EXPERIMENTAL METHOD

Experiments were carried out on (C57BL/6 × A/Sn)F_1 mice weighing 20-22 g, obtained from the Rappolovo Nursery, Academy of Medical Sciences of the USSR. *M. arthritidis* was obtained as described previously [3]. Mice were infected intraperitoneally with *M. arthritidis* in a dose of 10^8 CFU in 0.5 ml physiological saline. The 5-FU (from Sigma, USA) was injected intraperitoneally in a dose of 150 mg/kg.

To estimate erythropoiesis quantitatively, ^{59}Fe citrate (specific activity 0.2 mCi/ml) was injected into mice in a dose of 0.5 μCi in 0.5 ml physiological saline. The mice were

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