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Immunobiol., vol. 172, pp. 143–150 (1986)

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The Effect of Glucocorticosteroids on Bone Marrow Mononuclear Phagocytes in Culture

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Received November 25, 1985 · Accepted in Revised Form April 25, 1986

Abstract

The effect of hydrocortisone and dexamethasone both *in vivo* and *in vitro* was studied in mouse bone marrow cultures in methylcellulose and in two liquid culture systems, one using Leighton tubes with a flying coverslip to grow adherent colonies and the other using Teflon culture bags to obtain suspension cultures. Although the total number of nucleated bone marrow cells was not greatly influenced by glucocorticosteroid treatment of the mice, a marked decrease in the number of colony-forming units and of mononuclear phagocytes was observed.

Inhibition of colony growth in methylcellulose and of growth of mononuclear phagocytes in Teflon culture bags also occurred when glucocorticosteroids were added to *in vitro* cultures. Both drugs caused an almost complete inhibition of the growth of adherent colonies, and ^3H -thymidine labeling of the cells was correspondingly low. When the glucocorticosteroids were added to cultures pre-incubated for 5 days in the presence of conditioned medium, the ^3H -thymidine labeling of macrophages and promonocytes was markedly reduced, whereas there was no change in the labeling of monoblasts.

Introduction

Glucocorticosteroids influence host defense mechanisms mainly by their action on the kinetics of lymphocytes, neutrophilic and eosinophilic leukocytes, and mononuclear phagocytes (1–6). In the mononuclear phagocyte series, monocytopenia is the most pronounced effect (1, 3). The exact mechanism by which monocytopenia is brought about is not known; earlier studies done in our laboratory suggested that a limited inhibition of monocyte production, a decreased release of monocytes from the bone marrow, and an increased disappearance of monocytes from the circulation probably all contribute to the observed monocytopenia (16, 17). The fact that most of these studies were done at a time when little was known about the proliferation of mononuclear phagocytes in the bone marrow compartment, led us to investigate the effect of glucocorticosteroids on bone marrow mononuclear phagocytes in more detail.

Materials and Methods

Animals

The study was performed in male Swiss mice weighing 25–30 g (Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, The Netherlands); the effect of glucocorticosteroids *in vitro* was studied in cells from specific pathogen-free Swiss mice, and the experiments on the *in vivo* effect were performed in Swiss mice with colonization-resistant flora (CRF mice) (7).

Bone marrow culture

The techniques used to harvest and culture bone marrow cells from the femur have been described in detail by GOUD et al. (8). The culture medium consisted of Dulbecco's modified Eagle's medium (Grand Island Biologicals, Grand Island, NY, U.S.A.) containing 20 % horse serum (Flow Laboratories, Irvine, Scotland) and 20 % conditioned medium. The conditioned medium was prepared with fibroblasts of embryonic mice, as described elsewhere (8, 9). This conditioned medium rather selectively promotes the growth of mononuclear phagocytes; the concentration of CSF 1 in this conditioned medium is of the order of 1500 U/ml (9). Batches of about the same strength were used throughout the experiments. The bone marrow cells were cultured in three ways:

- (1) in methylcellulose (10) according to the modification of GOUD et al. (8); colonies were counted under an inverted microscope after 4 and 7 days of incubation.
- (2) in the Teflon culture bag (TCB) (11). After 7 days of incubation, the cells were recovered from these liquid cultures and counted as described (9); cytocentrifuge preparations were made and stained with Giemsa stain or with α -naphthylbuturate esterase (12) to facilitate differential counting; and
- (3) in Leighton tubes with a flying coverslip, as described elsewhere (8, 9); in these liquid cultures, monoblasts, promonocytes, and macrophages were distinguished according to the criteria formulated by GOUD et al. (8). DNA synthesis was assessed by adding 0.1 μ Ci/ml 3 H-thymidine to the culture medium for 1 h and performing autoradiography as described elsewhere (9, 12).

All cultures were held at 37°C in a water-saturated atmosphere of 10 % CO₂ in air.

Glucocorticosteroids

For the studies on the effect of glucocorticosteroids *in vivo*, a depot of hydrocortisone acetate (Merck Sharp & Dohme, Haarlem, The Netherlands) was applied subcutaneously in a dosage of 15 mg. Dexamethasone sodiumphosphate (Organon, Oss, The Netherlands) was given in a dosage of 25 μ g subcutaneously in either one injection or in two injections 3 h apart. These dosage schedules were based on previous studies in our laboratory (1, 2). Per experiment at least 3 mice were used for the *in vitro* studies; hydrocortisone hemisuccinate (Merck Sharp & Dohme) or dexamethasone (Organon) was added to the cultures in various concentrations.

Results

In SPF mice treated with either hydrocortisone or dexamethasone, the numbers of bone marrow cells per femur, of colony-forming units in culture, and of mononuclear phagocytes in cultures were highly variable. More consistent results were obtained with CRF mice. The numbers of nucleated bone marrow cells after one dose of hydrocortisone and one or two dosages of dexamethasone, expressed as the percentage of the untreated control, are given in Figure 1. These results show that the total numbers of

nucleated bone marrow cells are not greatly influenced during the first 6 h of treatment, but in hydrocortisone-treated mice the numbers of cells after 24 h are significantly lower ($p < 0.02$; analysis of variance). In methylcellulose cultures, however, the number of colonies is decreased by more than 20% when the bone marrow had been sampled 6 h after glucocorticosteroid administration (Fig. 2A). The reduction of the number of colonies was more pronounced ($p < 0.02$) when the bone marrow was sampled 24 h after the administration of hydrocortisone. For dexamethasone, the differences are small and non-significant ($0.10 < p < 0.20$). In methylcellulose cultures of bone marrow cells from untreated CRF mice, between 60 and 85 colonies were found after 7 days incubation of 5×10^4 nucleated cells, and the majority of the colonies were mononuclear phagocyte colonies. Differential counting of cells is difficult in these colonies in semisolid medium.

In liquid cultures in TCBs of bone marrow of untreated CRF mice, $1.7\text{--}2.5 \times 10^5$ mononuclear phagocytes were found 7 days after the incubation of 2×10^5 nucleated bone marrow cells per ml. After glucocorticosteroid administration, this number decreased distinctly (Fig. 2B); the decrease in the number of mononuclear phagocytes 24 h after hydrocortisone is significant ($p = 0.05$); the differences after two doses of dexamethasone are also significant ($p = 0.01$).

The effect of glucocorticosteroids was also studied *in vitro*. When bone marrow cells of SPF mice were incubated in methylcellulose in the presence

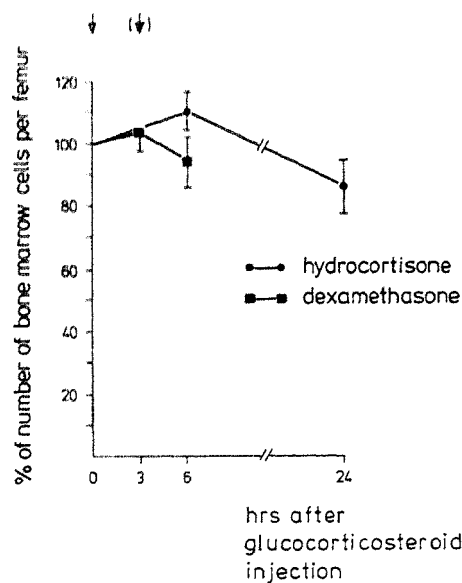


Fig. 1. The effect of one (↓) subcutaneous injection of 15 mg hydrocortisone and of one (↓) and two (↓↓) subcutaneous injections of 25 μg dexamethasone on the number of nucleated bone marrow cells per femur in CRF mice. The results are given as a percentage of the untreated controls. The bars represent standard error of the mean ($n = 10$).

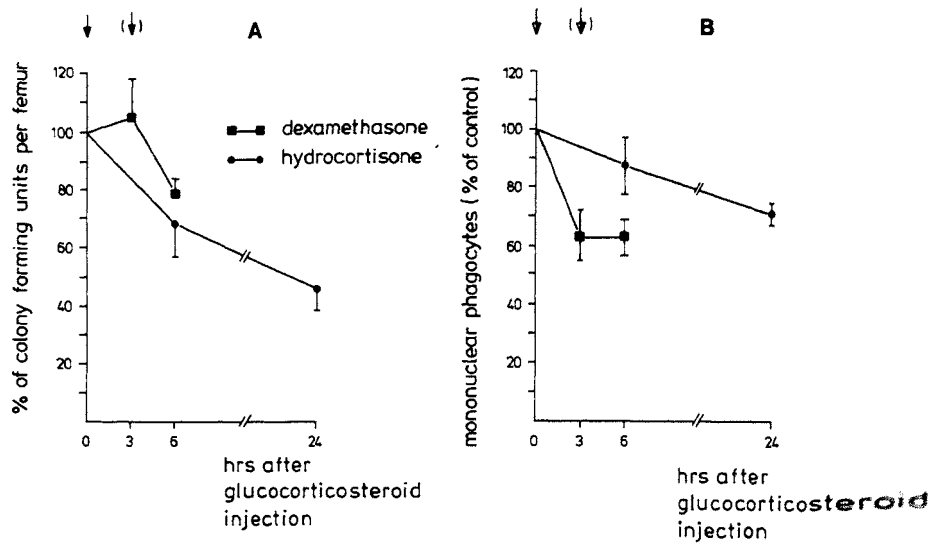


Fig. 2. The effect of one subcutaneous injection (\downarrow) of 15 mg hydrocortisone and of one (\downarrow) and two ($\downarrow\downarrow$) subcutaneous injections of 25 μ g dexamethasone on the number of colony forming units in bone marrow, determined in 7-day cultures in methylcellulose (panel A) and on the number of mononuclear phagocytes in 7-day bone marrow cultures in TCB (panel B). The results are given as a percentage of the untreated controls. The bars represent standard error of the mean ($n = 4$).

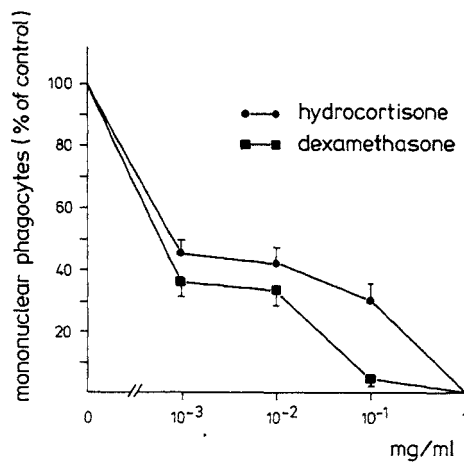


Fig. 3. The effect of various concentrations of hydrocortisone and dexamethasone added to cultures of bone marrow mononuclear phagocytes of SPF mice in TCB. The cultures were incubated for 7 days. The results show a dose-dependent effect on the number of mononuclear phagocytes. The bars represent standard error of the mean ($n = 4$).

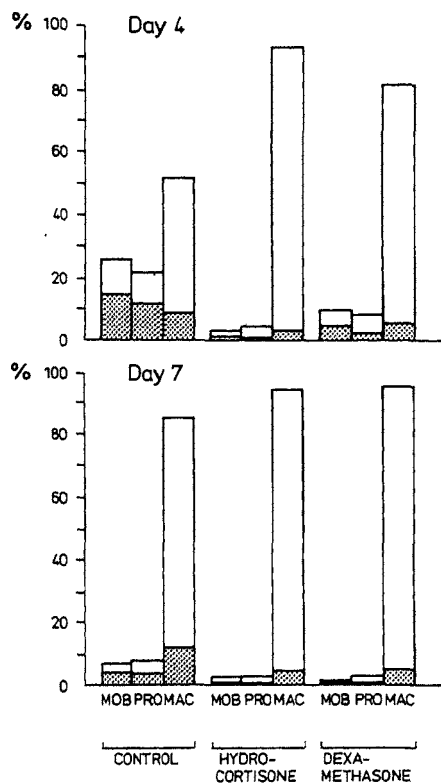


Fig. 4. Differentiation (open bars) and 1-h ³H-thymidine labeling (stippled bars) in bone marrow colonies cultured for 4 and 7 days in the presence of 10⁻² mg/ml hydrocortisone or dexamethasone in Leighton tubes with a coverslip. The bone marrow originated from SPF mice. mob = monoblasts, pro = promonocytes, mac = macrophages.

of 10⁻² mg/ml hydrocortisone hemisuccinate for 7 days, the number of colonies amounted to 41.8% (SD ± 6.1%) of the number in control cultures. In 7-day liquid cultures in TCBs, a dose-dependent effect of both hydrocortisone and dexamethasone on the formation of mononuclear phagocytes was observed (Fig. 3). At concentrations of 10⁻² to 10⁻³ mg/ml of either glucocorticosteroid, inhibition of mononuclear phagocyte production amounting to more than 50% was seen *in vitro*. In cultures on glass coverslips, the growth of mononuclear phagocytes was even more strongly inhibited: at 10⁻² mg/ml hydrocortisone or dexamethasone, colony formation was virtually abolished. In these coverslip cultures, the total number of cells was strongly reduced compared to control cultures, and the cells were scattered over the glass surface; there were few monoblasts and promonocytes, and the majority were macrophages (Fig. 4). The number of cells in the supernatant did not differ from the controls (data not shown). The poor growth of mononuclear phagocytes was reflected by very low 1 h ³H-thymidine labeling indices (Fig. 4).

Table 1. ³H-Thymidine labeling of mononuclear phagocytes incubated with glucocorticosteroids^a

	Control %	Hydrocortisone ^b %	Dexamethasone ^b %
Monoblasts	69	71	73
Promonocytes	41	22	32
Macrophages	34	24	24

^a incubated for 24 h after culture for 5 days^b 10⁻² mg/ml

To study the effect of glucocorticosteroids once colonies have formed, 5-day cultures were exposed to 10⁻² mg/ml hydrocortisone or dexamethasone for 24 h. In these experiments the percentages of monoblasts, promonocytes, and macrophages did not alter in the presence of glucocorticosteroids, but a consistent change in the ³H-thymidine labeling indices was found; after incubation with hydrocortisone or dexamethasone, the labeling indices of promonocytes and macrophages were distinctly reduced, whereas the labeling of monoblasts was not affected. The results of a representative experiment are given in Table 1.

Discussion

Glucocorticosteroids have been shown to regulate the proliferation of many kinds of cell (13–18). A number of studies have demonstrated an inhibitory effect of glucocorticosteroids on bone marrow cultures (13, 17–21). Because semisolid media were used in most of these studies, the information they provide on the effects on proliferation of mononuclear phagocytes is limited. An exception is the study of NEUMANN and SORG (21) in which a liquid culture system is used; they found inhibition of proliferation of bone marrow mononuclear phagocytes. In the present study, significant effects of glucocorticosteroids on the production of bone marrow mononuclear phagocytes were found using various culture systems. Thus, inhibition of the production of these cells in the bone marrow compartment is probably greater than was originally thought (1, 2).

Mice injected with hydrocortisone or dexamethasone showed a small decrease in the number of nucleated bone marrow cells and a much greater reduction of the number of colonies grown from bone marrow sampled within 24 h after the injection. This finding differs slightly from that of METCALF (20), who reported that the number of colonies did not decrease until three days after an injection of cortisone.

Although there are unexplained differences in the magnitude of the effects of glucocorticosteroids in the different culture systems used, our data point to an effect of these hormones at the level of the colony forming

unit, in all probability an effect on a committed stem cell. The experiments in which the glucocorticosteroids were added to the cultures showed strong inhibition of colony growth on glass accompanied by very low numbers of monoblasts and promonocytes, also indicating an effect on the precursors of the monoblast. The macrophages present in these cultures were probably cells that were already mature when plated.

The conclusion that the glucocorticosteroids act at the level of the committed stem cell of the mononuclear phagocyte series is supported by the findings of MOTOMURA et al. (21), who saw increased granulocytopoiesis and reduced monocytopoiesis in long-term bone marrow cultures exposed to hydrocortisone.

The maintenance of DNA synthesis in monoblasts after exposure of precultured bone marrow to glucocorticosteroids is remarkable. These results can be interpreted in two ways: either the monoblasts are not affected by the glucocorticosteroids or they are arrested in the late S phase of the cell cycle. The latter possibility is not likely in view of reports that in other cell types glucocorticosteroids lead to an arrest in the G1 phase (23, 24).

Our highly variable results in the *in vivo* experiments using a regular SPF mouse strain and the better results with CRF mice are in accordance with the observations of VAN WAARDE et al. (7), showing that monocytopoiesis, as reflected by blood monocyte counts, varies with the condition of the mice, i.e., whether they are infected. It is not known what stimuli of monocytopoiesis are operative in these mice.

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