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The Effect of Hydrocortisone Acetate and Azathioprine (Imuran) on the Kinetics of Neutrophilic Polymorphonuclear Leucocytes during an Acute Inflammation (39593)

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Glucocorticosteroids and azathioprine have both an antiinflammatory and an immunosuppressive effect. This is reflected, for example, in an altered composition of the inflammatory exudate. The mechanism underlying the action of these drugs is not the same, however.

Several mechanisms have been suggested to explain the effect of glucocorticosteroids on the cellular composition of inflammatory exudates. One of these concerns an influence on the vascular wall leading to decreased permeability, thus preventing the passage of leukocytes immigrating to the site of inflammation (1-5). The effect has also been attributed to a decreased motility of the leukocytes (6, 7). A third mechanism could be based on a lower number of circulating leukocytes available for the formation of the inflammatory exudate (8-10).

Azathioprine is an antimetabolite with an effect on DNA and RNA synthesis, thus inhibiting cellular proliferation and protein synthesis (11, 12). The change in the composition of the cellular exudate during an inflammatory response could be due to an inhibition of leukocyte production in the bone marrow, causing a decrease in the number of circulating leukocytes, and thus fewer cells are available to migrate to the site of the lesion (13-20).

Both glucocorticosteroids and azathioprine have as a side effect a decreased host resistance to invading microorganisms, which may be due to either an altered number or function of phagocytic cells (i.e., granulocytes and mononuclear phagocytes), or an effect on the number or function of lymphocytes (21, 22), or on the interaction between lymphocytes and mononuclear phagocytes (23, 24). The effect of glucocorticosteroids (25, 27) and azathioprine (19, 20) on mononuclear phagocytes has been studied in detail, and their effect on lymphocytes is known to some extent (21, 22), but the effect on the granulocytes during an inflammatory response remains uncertain.

The present paper reports the effects of glucocorticosteroids and azathioprine on the number of granulocytes in the circulation and in the peritoneal cavity of mice during the normal steady state and during an acute inflammatory reaction.

Materials and methods. The materials and methods were essentially similar to those described earlier (19, 25).

Animals. In the present studies, specific pathogen-free male Swiss mice, weighing 25-30 g (Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, Holland), were used.

Blood leukocyte counts. Leukocyte-counting was done in a Bürker hemocytometer; the blood was diluted 1:20 in a leukocyte pipet with Türk's solution, which contains 6% acetic acid. Differential counts were done on 200 leukocytes from blood smears fixed in absolute methanol and stained with the Giemsa stain. From these data, the number of cells was calculated for each category of leukocytes.

Peritoneal cell counts. The peritoneal cells were washed out, under strictly standard conditions, with 2 ml of phosphate-buffered saline (pH 7.2) (Difco Laboratories, Inc., Detroit, Mich.) containing heparin (50 units/ml).

Total leukocyte counts were done in undiluted, unstained cell suspensions in a Bürker hemocytometer. In the inflammation experiments, the cells were sometimes diluted 1:10 in a 0.2% trypan blue solution in saline. Differential counts were done in preparations made with a sedimentation apparatus. The cells sedimented on a microscope slide were fixed in absolute methanol and stained with Giemsa stain. These data were

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used to calculate the number of peritoneal granulocytes per milliliter of washout fluid. The peritoneal cavity of normal mice contains virtually no fluid, and the recovery of intraperitoneally injected saline is variable and always less than complete. Therefore the number of cells per milliliter is the best measure of the cellular population of the peritoneal cavity.

Drugs. Hydrocortisone acetate (kindly provided by Merck, Sharp and Dohme, Haarlem, The Netherlands) was injected sc in the nuchal region, giving a depot from which a steroid effect was sustained for at least 14 days (25). Azathioprine (Imuran, kindly provided by Burroughs Wellcome Co., London, England) was dissolved in pyrogen-free distilled water, and 0.5 ml of the appropriate dilution was injected sc in the nuchal region.

Inflammation. An acute inflammatory reaction was induced by a single injection of 1 ml of sterile newborn calf serum (NBCS, Grand Island Biological Co., Grand Island, N.Y.) into the peritoneal cavity (28, 29).

General remarks. Each animal was used only once for leukocyte and peritoneal cell sampling. Mice showing a hemorrhagic exudate were discarded. The results given for all time points represent the mean value of at least four animals.

Results. The course of the number of neutrophilic polymorphonuclear leukocytes in the peritoneal cavity and the peripheral blood during an acute inflammation. In normal animals the peritoneal cavity contains 1–2 × 10^4 polymorphonuclear leukocytes per ml, which is 0.5 to 1% of the total number of peritoneal leukocytes. From 2 hr after an ip injection of 1 ml of NBCS, the increase in the amount of fluid is no more than 0.1 ml, because NBCS is rapidly absorbed (30). During the inflammatory reaction, the number of granulocytes in the peritoneal cavity increases rapidly to a maximum of 197 × 10^4 cells/ml at 6 hr; thereafter, the granulocytes disappear rapidly (Figs. 2 and 5).

The peripheral blood of untreated mice contains 1400 granulocytes per mm^3 (range 360–4480). During an acute inflammatory response induced by NBCS, the number of peripheral blood granulocytes increases until a peak level of (4800–6000 cells/mm^3) is reached at 6 hr (Fig. 1), and then levels off to normal values at 24 hr.

The effect of hydrocortisone acetate on the number of neutrophilic polymorphonuclear leukocytes in the peritoneal cavity and the peripheral blood during an acute inflammation. In animals treated with 15 mg of hydrocortisone acetate, the number of peripheral blood granulocytes increases slightly during the first 6 days (Fig. 1) and the number of granulocytes in the peritoneal cavity remains low. When an acute inflammation is evoked in these hydrocortisone-treated animals, the number of peritoneal granulocytes increases to a maximum of 426 × 10^4 cells/ml at 6 hr, a higher level than that in the control group, and then decreases rapidly (Fig. 2). The number of granulocytes in the circulation also increases to a maximum (6915 cells/mm^3) at 6 hr and then levels off (Fig. 1).

The effect of azathioprine on the number of neutrophilic polymorphonuclear leukocytes in the peritoneal cavity and the peripheral blood during an acute inflammation. Treatment with azathioprine affects the number of granulocytes in the circulation; the effect is related to the dose applied (Fig. 3).

In animals pretreated for 4 days with 200 mg/kg of azathioprine, an ip injection of NBCS leads to an increase in the number of neutrophilic polymorphonuclear leukocytes in the peritoneal cavity with a peak value at

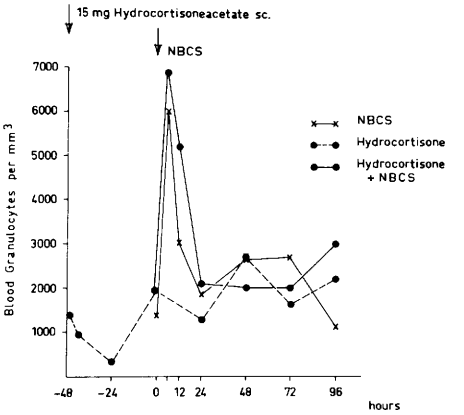

FIG. 1. Course of the number of peripheral blood granulocytes during an acute inflammatory reaction in the peritoneal cavity during treatment with hydrocortisone acetate and in control mice.

6 hr (140×10^4 cells). When the animals were pretreated with 3 mg/kg of azathioprine, the response of the polymorphonuclear leukocytes in the peritoneal cavity was only slightly (10%) lower (210×10^4 cells/ml at 12 hr) than that in the untreated animals (Fig. 5).

After treatment for 4 days with 200 mg/kg of azathioprine, the number of granulocytes in the circulation decreases to 660/mm^3; an ip injection of newborn calf serum in these animals causes an increase in the number of neutrophilic polymorphonuclear leukocytes to 3535/mm^3 at 6 hr, but thereafter the number of granulocytes drops rapidly (Fig. 4). Animals treated with a lower dose of azathioprine (3 mg/kg) showed no decrease in the number of peripheral blood granulocytes. When these animals received an inflammatory stimulus, the granulocytes increase to a peak value of 5660 granulocytes/mm^3 at 6 hr. Initially, this response is similar to that in normal mice, but at later time points, the number of granulocytes is lower in treated than in untreated animals (Fig. 4).

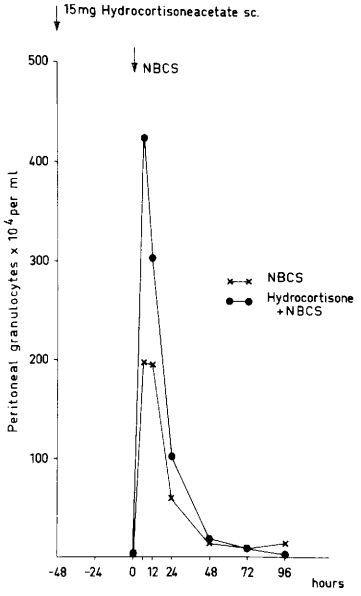

FIG. 2. Course of the number of granulocytes in the peritoneal cavity during an acute inflammatory reaction induced by an ip injection of newborn calf serum in mice treated with hydrocortisone acetate and in control mice.

Discussion. The main conclusion to be drawn from this study is that neither hydrocortisone acetate nor azathioprine causes a significant reduction in the number of neutrophilic granulocytes in the exudate of an acute inflammatory lesion.

Hydrocortisone increases the number of circulating granulocytes, as found earlier (3), which implies that granulocytes are available to migrate into the site inflammation. The course of the peripheral blood neutrophils during the acute inflammatory response indicates that new cells are also recruited from the bone marrow into the peripheral blood. Since the number and rate of appearance of granulocytes at the site of the lesion is not decreased by hydrocortisone, it is unlikely that vascular permeability for the granulocytes or the motility of these cells is affected either.

The effect of hydrocortisone on the neutrophils differs widely from that on the mononuclear phagocytes, since this drug causes a severe monocytopenia in the

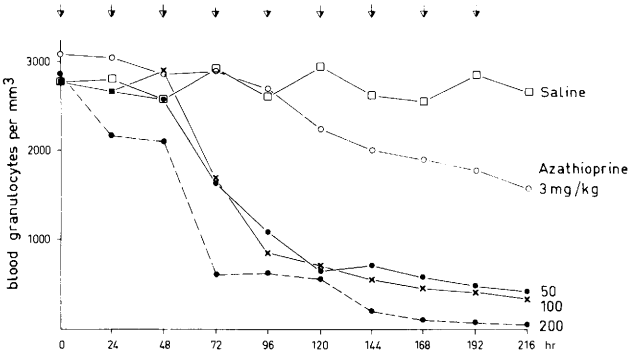

FIG. 3. The effect of treatment with various doses of azathioprine on the number of peripheral blood granulocytes.

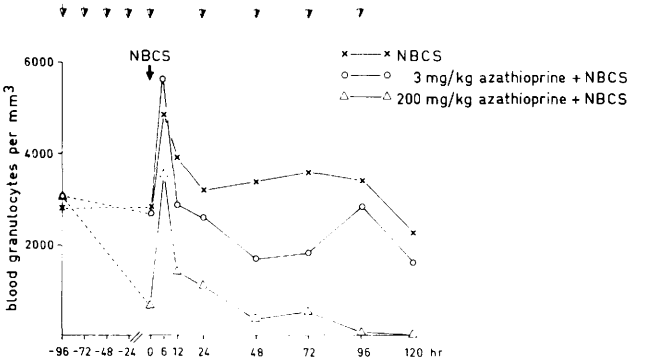

FIG. 4. Course of the number of peripheral blood granulocytes during an acute inflammatory reaction in the peritoneal cavity under treatment with azathioprine and in control mice.

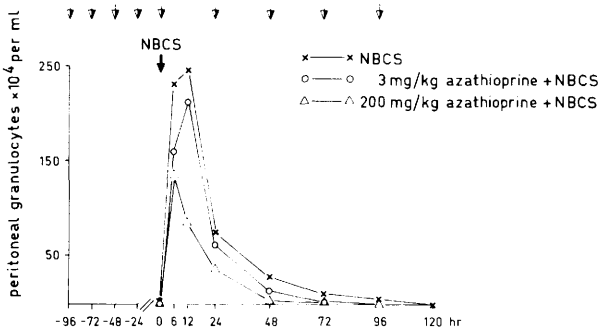

FIG. 5. Course of the number of granulocytes in the peritoneal cavity during an acute inflammatory reaction caused by an ip injection of newborn calf serum in mice treated with azathioprine and in control mice.

mouse, the rat, the guinea pig, the rabbit, and man (25, 32–35); and the absence of an increase in the number of mononuclear phagocytes in the inflammatory exudate is explained by the severe monocytopenia that cannot be overcome by the inflammatory stimulus (25).

The action of azathioprine is quite different. This drug inhibits the proliferation of the granulocytes' precursors in the bone marrow, thus bringing about a decrease in the circulating granulocytes after 4 days of treatment with a high dose of the drug. However, the stimulus of an acute inflammation induces the release of a sufficient number of (mature) granulocytes from the bone marrow pool into the peripheral blood, which then migrate to the site of the lesion. Treatment with a low dose, which is about equivalent to a nontoxic, immunosuppressive, antiinflammatory dose in man (19), does not affect the circulating granulocyte level, however, and does not impair the granulocyte response during an acute inflammation.

It is of interest that the effect of azathioprine on the neutrophilic polymorphonuclear leukocytes differs from that on the mononuclear phagocytes. A low dose of this drug causes a reduction in the number of circulating monocytes, and the influx of mononuclear phagocytes into the site of inflammation is significantly shorter and smaller than in the absence of azathioprine (19, 20).

It may be that the short duration of the neutrophil response during an acute inflammation masks a possible effect of hydrocortisone or azathioprine, since for such a short response enough granulocytes are still available in the bone marrow reserve pool. However, if there is a sustained need for granulocytes during treatment with these drugs, too few cells might be available and the granulocytes' response will be abnormal.

Summary. The course of the number of

neutrophilic polymorphonuclear leukocytes during an acute inflammation in the peritoneal cavity was studied in mice treated with hydrocortisone or azathioprine. The results show that in these animals the number of neutrophilic granulocytes appearing in the inflammatory exudate is similar to that in animals not treated with these antiinflammatory drugs. This study indicates that glucocorticosteroids do not affect the vascular permeability for granulocytes or the motility of these cells, and that during azathioprine treatment the bone marrow contains a sufficient number of neutrophils that can be released into the circulation.

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