The Effect of Azathioprine (Imuran) on the Kinetics of Monocytes and Macrophages During the Normal Steady State and an Acute Inflammatory Reaction

By Andreas E. Gassmann and Ralph van Furth

The effect of azathioprine on the kinetics of peripheral blood monocytes and peritoneal macrophages was studied in normal mice and in mice in which an inflammatory reaction was provoked. Two dosage levels were used: a high dose of 200 mg/kg which is the maximum tolerated daily dose in mice, and a low dose of 3 mg/kg which is about equivalent to a nontoxic, immunosuppressive, anti-inflammatory dose in man. The number of peripheral blood monocytes decreases gradually during azathioprine treatment of normal mice, the extent and duration being dependent on the dose and duration of administration of the drug: a high dose administered over a period of 9 days gives an almost complete reduction, and a low dose (3 mg/kg) given for the same period results in a reduction of about 50%. This effect seems to be reversible, because when treatment is stopped the number of monocytes starts to increase 24-48 hr later. The number of peritoneal macrophages is only affected when a high dose (200 mg/kg) is given over a long period; a low dose has virtually no effect. In mice in which an inflammatory reaction was provoked in the peritoneal cavity, the normally occurring increase in the numbers of both peripheral blood monocytes and peritoneal macrophages was suppressed, the extent being dependent on the dose of azathioprine administered. Labeling studies with ³H-thymidine indicated that the reduction of peripheral blood monocytes and peritoneal macrophages in the inflammatory exudate is due to a diminished monocyte production.

MONONUCLEAR PHAGOCYTES form a cell line that originates in the bone marrow. The initial cell, the monoblast, gives rise to promonocytes, which in turn are the precursors of the monocytes. The monocytes are transported by the peripheral blood and ultimately become tissue macrophages.¹,² It has been demonstrated that this pathway holds not only for the normal steady state but also during an acute inflammatory response.³,⁴ In the latter state, the kinetics change: the production of monocytes in the bone marrow is increased, as is the number of monocytes in the peripheral blood, and the inflammatory exudate contains more macrophages.⁵

6-Mercaptopurine and its derivative azathioprine (Imuran) can alter both the immunologic and nonimmunologic host defense mechanisms. These antimetabolites interfere with the synthesis of nucleic acid (RNA and DNA),⁶ thus inhibiting protein synthesis and cell proliferation. These compounds are widely used clinically because they inhibit immunoglobulin and (auto-) anti-
body synthesis, suppress cell-mediated immunity, and delay the rejection of foreign tissue grafts (see review by Bach, 1975). Both drugs also have an anti-inflammatory effect, since they reduce cellular infiltration at the site of a lesion.

The important role played by the mononuclear phagocytes in both immunologic reactions and the inflammatory response raised the question of whether 6-mercaptopurine or azathioprine would affect the production and kinetics of such cells and therefore have an influence on the mechanisms involved in host defense.

The present report describes the effect of azathioprine on the kinetics of peripheral blood monocytes and peritoneal macrophages during the normal steady state and an acute inflammatory reaction.

MATERIALS AND METHODS

Animals
This investigation was done in specific pathogen-free male Swiss mice (25-30 g) obtained from the Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, The Netherlands.

Azathioprine
Azathioprine (Imuran, 50-mg vials, freeze-dried) was kindly provided by the Burroughs Wellcome Company, London, England. Immediately before use, 5 ml pyrogen-free distilled water was added to the vial. When necessary, further dilutions were made such that the volume to be injected was always 0.5 ml. The drug was administered subcutaneously in the nuchal region at intervals of 24 hr unless otherwise stated. The doses given were 200, 100, 50, or 3 mg per kg body weight.

6-Mercaptopurine
6-Mercaptopurine (Burroughs Wellcome Co.) was used only for in vitro experiments. A fresh stock solution (3000 µg/ml) was prepared daily by dissolving 6-mercaptopurine in 0.1 ml 1 N NaOH and 0.9 ml medium 199 (Microbiological Associates, Inc., Bethesda, Md.). The stock solution was further diluted with medium 199 before addition to the culture medium.

Inflammation
An inflammatory reaction was provoked by a single injection of 1 ml sterile newborn calf serum (NBSC) (Grand Island Biological Co., Grand Island, N.Y.) given intraperitoneally with a small needle (Yale, 26 G 3/8, Becton and Dickinson Co., Ltd., Dublin, Ireland). Mice showing a hemorrhagic exudate were discarded.

Blood Leukocyte Counts
Blood taken from tail veins (two samples per animal) was diluted 1:20 with Türk's solution containing 6%, acetic acid, and the leukocytes were counted in a Bürker hemocytometer in duplicate. Blood smears for differential counts were immediately air dried, fixed in absolute methanol, and stained with Giemsa stain. Differential counts were done on 100 leukocytes from at least two blood smears, and the number of cells per cubic millimeter was calculated for each leukocyte category.

Peritoneal Cell Counts
Mice were killed rapidly by chloroform inhalation and the skin stripped from the abdomen. Peritoneal cells were obtained by injecting 2 ml phosphate-buffered saline (pH 7.2, Difco Laboratories, Inc., Detroit, Mich.) containing 50 U/ml heparin into the peritoneal cavity, kneading the abdomen gently, and removing the cell suspension with a capillary pipette after 1 min. The fluid
was collected in a sterile plastic tube (Falcon Plastics, Gateway International, Los Angeles, Calif.), and cell counts were done in duplicate on the undiluted suspension without staining. Differential counts were performed in preparations made on microscope slides with a sedimentation apparatus, fixed in absolute methanol, and stained with Giemsa stain. For each animal, at least 200 consecutive cells were identified and the numbers of peritoneal macrophages and lymphocytes per milliliter calculated. In the inflammation experiments, this was also done for the polymorphonuclear leukocytes.

**Cell Cultures**

The suspension of peritoneal cells was centrifuged for 8 min at 110 g at room temperature. The cells were then resuspended in 2 ml culture medium consisting of medium 199 (Microbiological Associates Inc., Bethesda, Md.), 20% newborn calf serum (Grand Island Biological Co., Grand Island, N.Y.) inactivated at 56°C for 30 min, and 200 U/ml penicillin G. The resulting suspension was incubated in a Leighton tube (Bello Glass Inc., Vineland, N.J.) containing a flying cover slip, in an atmosphere of 5% CO2-air mixture at 37°C for 2 or 24 hr. After incubation, the medium was removed and the cover slip washed three times with medium 199, fixed in methanol, air dried, and stained with Giemsa stain. For radioautography, the cover slips were fixed in methanol, washed immediately in three changes of distilled water over a 12-hr period, and then air dried.

**Labeling Studies**

In vivo labeling was done with a single intravenous injection into a tail vein or four intramuscular injections (at 3-hr intervals, into alternate hind legs) of 1 μCi per g body weight of 3H-thymidine (specific activity, 6.7 Ci/m mole; New England Nuclear Corporation, Boston, Mass.). The in vitro labeling of peritoneal macrophages was done by incubating the cells in a culture medium containing 0.1 μCi/ml 3H-thymidine.

**Radioautography**

Radioautography was performed with Ilford Nuclear Research emulsion K5 in gel form (Ilford Ltd., Ilford, Essex, England). The exposure time was 21 days. The slides were developed with Kodak Developer D-19 (Kodak Ltd., London, England) and stained with Giemsa stain at pH 5.7. All cells with three or more silver grains over the nucleus were considered as labeled, since preparations from nonlabeled animals showed less than three grains per nucleus.

**Identification of the Cells**

The morphologic criteria used for the identification of the peripheral blood monocytes and peritoneal macrophages have been described elsewhere.18,19

**General Remarks**

Each time point on the graphs represents the mean value of at least four animals. Each animal was only used once. The difference between the control and treated animals was tested for significance with the Student's t test.

**RESULTS**

**The Effect of Azathioprine on the Number of Peripheral Blood Leukocytes**

The effect of azathioprine on the number of leukocytes in the peripheral blood and peritoneal cavity must be known before the effect of this drug on an acute inflammatory response can be studied. Daily injection of 200 mg/kg azathioprine for a period of 4 days gives a gradual decrease in the number of peripheral blood leukocytes. The lowest values of the granulocyte (441 cells per cu mm) and lymphocyte (2685 cells per cu mm) counts reached at 96 hr (Fig. 1) differ significantly from the initial value (p < 0.01). The number of lympho-
cytes started to increase 2 days after the last azathioprine injection; the increase of granulocytes occurred 24 hr later.

The monocytes reached their lowest level of 32 cells per cu mm, at 96 hr (i.e., 10% of the initial value, a difference that is highly significant ($p < 0.001$)), and their number increased gradually from 120 hr onward. The normal level, however, was not yet reached 5 days after the last azathioprine administration.

![Graph showing the effect of daily administration of 200 mg/kg azathioprine on the number of peripheral blood monocytes, granulocytes, and lymphocytes.](image1)

**Fig. 1.** The effect of daily administration of 200 mg/kg azathioprine (•) on the number of peripheral blood monocytes (Δ—Δ), granulocytes (×—×), and lymphocytes (○—○).

![Graph showing the effect of long-term treatment with azathioprine in various dosages on the number of peripheral blood monocytes.](image2)

**Fig. 2.** The effect of long-term treatment with azathioprine in various dosages on the number of peripheral blood monocytes.
Administration of 200 mg/kg azathioprine as an emulsion in 2% methylcellulose (400 cps without conservant) for slower release of the drug, or administration at shorter intervals (100 mg/kg every 12 hr), led to a similar decrease in the number of monocytes, but the recovery was slower. The level lay at 90 cells per cu mm 6 days after the termination of administration of 100 mg/kg azathioprine at 12-hr intervals.

The effect of various dosage schedules over a longer period was studied next (Fig. 2). Treatment with 200 mg/kg azathioprine for 9 days gave a gradual decrease in the number of monocytes to a value of 1 per cu mm at 216 hr; the difference from the control group was highly significant \( p < 0.001 \). Lower doses of azathioprine also led to a gradual reduction in the number of monocytes. With the low dosage (3 mg/kg azathioprine), the peripheral blood showed at the same time point 149 monocytes per cu mm; this was about 50% of the value found in untreated mice, a difference that was statistically significant \( p < 0.01 \). When treatment with 3 mg/kg azathioprine was prolonged for 20 days, the number of monocytes continued to decrease, reaching 40% of the normal value. In a control experiment, subcutaneous injections of buffered saline had no effect on the number of monocytes (Fig. 2).

**The Effect of Azathioprine on the Number of Peritoneal Macrophages and Peritoneal Lymphocytes**

When 200 mg/kg azathioprine was given for 4 days, no reduction in the number of peritoneal macrophages or peritoneal lymphocytes occurred. The same dose of azathioprine suspended in 2% methylcellulose also had no effect. Prolonged administration of 200 mg/kg azathioprine (9 days) led to a gradual decrease in the number of peritoneal macrophages, the level reaching 69 \( \times 10^4 \) per ml (about 50% of the normal value) 24 hr after the last injection (Fig. 3). This decrease during the period of 9 days was statistically highly significant \( p < 0.001 \). The number of lymphocytes also decreased to 55% of the normal value at that time point.

The administration of azathioprine in a dose of 3 mg per kg body weight over a period of 9 days induced virtually no reduction (less than 5%) in the number of peritoneal macrophages (Fig. 3) or lymphocytes.

**The Effect of Azathioprine on the Number of Peritoneal Macrophages and Peripheral Blood Monocytes During an Acute Inflammatory Reaction**

**Peritoneal macrophages.** In mice an intraperitoneal injection of 1 ml sterile newborn calf serum gave an initial decrease followed by a rise in the number of macrophages in the peritoneal cavity, reaching a maximum (i.e., \( 273 \times 10^4 \) cells per ml or two and one-quarter times the normal value) after 72 hr (Fig. 3). Simultaneously, the number of monocytes in the circulation increased, the maximum (748 cells per cu mm or two and one-quarter times the normal value) being reached 48 hr after the intraperitoneal injection (Fig. 4).

When this kind of inflammation was induced in animals treated with azathioprine, the inflammatory reaction ran a different course. After 4 days of pretreatment with 200 mg/kg azathioprine, the inflammatory stimulus caused an initial decrease followed by an increase in the number of peritoneal macro-
Fig. 3. The effect of azathioprine on the number of peritoneal macrophages in normal mice and during an acute peritoneal inflammation. After daily treatment with 200 mg/kg azathioprine (+) for 9 days, the number of peritoneal macrophages is reduced by about 50%, whereas daily treatment with 3 mg/kg azathioprine has virtually no effect. An intraperitoneal injection of 1 ml NBCS (+) causes an increase in the number of peritoneal macrophages in normal mice. This effect is considerably smaller in mice treated with azathioprine.

Fig. 4. The effect of azathioprine (daily treatment with 3 or 200 mg/kg) (+) on the number of peripheral blood monocytes during an acute inflammation in the peritoneal cavity provoked with an injection of 1 ml NBCS (+). For comparison, the course of the number of peripheral blood monocytes in normal mice with a similar acute peritoneal inflammation is shown.
phages to $99 \times 10^4$ cells per ml, which was still lower than the number found in animals treated with azathioprine alone ($p < 0.001$) (Fig. 3).

When an acute inflammation was induced in animals treated for 4 days with a low dose of azathioprine (3 mg/kg), 24 hr after a NBCS injection the number of peritoneal macrophages was roughly doubled ($226 \times 10^4$ cells per ml), a reaction similar to that in animals not treated with azathioprine. After that, however, there was no further increase in the number of peritoneal macrophages as is usually found during an inflammatory reaction. Instead, there was a decrease, and at 72 hr the same level ($136 \times 10^4$ cells per ml) was seen as in unprovoked animals treated with 3 mg/kg azathioprine (Fig. 3). At this time the difference between the azathioprine-treated animals and the controls was highly significant ($p < 0.001$).

Peripheral blood monocytes. The monocytosis occurring during an acute inflammatory response to newborn calf serum was almost absent in azathioprine-treated animals also. Animals given the high dose of azathioprine (200 mg/kg) showed a slight increase from 62 to 91 monocytes per cu mm at 48 hr after the injection of NBCS (Fig. 4). In animals treated with 3 mg/kg azathioprine, the number of monocytes increased 12 hr after the NBCS injection from 223 cu mm (i.e., the monocyte level after 4 days of azathioprine treatment) to 292 per cu mm. The number of monocytes then showed no further increase but leveled off during the next 60 hr, reaching values similar to those seen in unprovoked animals treated with the same dose of azathioprine (Fig. 4). At 48 hr, the difference between animals treated with azathioprine and NBCS and those treated with NBCS alone was highly significant ($p < 0.001$).

The Effect of Azathioprine on the Kinetics of Labeled Peripheral Blood Monocytes and Peritoneal Macrophages

The effect of azathioprine on the course of labeled peripheral blood monocytes and peritoneal macrophages was studied in animals given four intramuscular injections of $^3$H-thymidine at 3-hr intervals and 3 hr after the last of these injections, the first of four daily azathioprine (200 mg/kg) injections.

In normal mice the labeling index of monocytes increased 12 hr after the last $^3$H-thymidine injection, from 59.5% to a maximum value of 82.5% at 60 hr (Fig. 5), which is in agreement with earlier findings. In the azathioprine-treated animals at 12 hr, the same labeling index (57.5%) was found as in normal mice (these animals had then only received one injection of azathioprine, 9 hr earlier). However, in these azathioprine-treated animals, the number of labeled monocytes remained almost constant during the next 24 hr and then dropped gradually (Fig. 5).

The study on peritoneal macrophages done in the same animals showed that in the normal mice the percentage of labeled macrophages increased from 5.4% at 12 hr to a maximum of 13.6% at 84 hr (Fig. 6), which agrees well with previous findings. In animals treated with azathioprine, the percentage of labeled peritoneal macrophages was 6.0 at 12 hr and did not increase after that, but remained almost constant (Fig. 6).

These experiments indicate that during azathioprine treatment fewer labeled mononuclear phagocytes enter the circulation and then reach the peritoneal
Fig. 5. The effect of 200 mg/kg azathioprine on the labeling index of peripheral blood monocytes of mice given four injections of $^3$H-thymidine during the 12 hr before zero time.

Fig. 6. The effect of 200 mg/kg azathioprine on the labeling index of peritoneal macrophages of mice given four injections of $^3$H-thymidine during the 12 hr before zero time.
KINETICS OF MONOCYTES AND MACROPHAGES

Table 1. Labeling of Blood Monocytes After Treatment With Azathioprine

<table>
<thead>
<tr>
<th>Time After 1H-thymidine Injection* (hr)</th>
<th>Percentage of Labeled Blood Monocytes</th>
<th>Total Number of Labeled Blood Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (%)</td>
<td>Azathioprine† (%)</td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>48.0</td>
<td>3.5</td>
</tr>
<tr>
<td>48</td>
<td>59.0</td>
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<td>72</td>
<td>42.0</td>
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</tr>
<tr>
<td>96</td>
<td>37.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

*10.1μCi/g 1H-thymidine intravenously simultaneously with the last azathioprine injection.
†Pretreatment for 4 days with 200 mg per kg body weight azathioprine daily.

cavity. To find out whether or not the absence of an increase in the number of labeled monocytes is due to diminished production, the following experiment was performed.

Animals pretreated for 4 days with 200 mg/kg azathioprine received one intravenous injection of 1H-thymidine simultaneously with the last dose of azathioprine, after which the percentage and total number of labeled monocytes were determined. The highest labeling index (12.5%) in azathioprine-treated animals occurred at 48 hr (Table 1). Although azathioprine treatment was stopped at that time point, the total number of monocytes remained low (32 per cu mm, see Fig. 1), and thus the number of labeled monocytes (4 per cu mm) was considerably lower than in normal animals (Table 1). The results of these labeling experiments strongly support the conclusion that azathioprine reduces the production of monocytes as reported in detail in the accompanying paper.

The Effect of Azathioprine on the Kinetics of Labeled Monocytes and Peritoneal Macrophages During an Acute Inflammation

The experiments described above demonstrate that in azathioprine-treated animals an inflammatory stimulus does not induce either monocytosis or an increase in the number of peritoneal macrophages, as occurs in normal animals. To obtain more information about the kinetics of monocytes and peritoneal macrophages during an acute inflammatory response, the course of labeled mononuclear phagocytes was studied in animals treated with a low dose of azathioprine.

Two groups of animals were given 3 mg/kg azathioprine for 9 days. After 4 days of treatment, all of the mice received a single injection of 1H-thymidine, and 1 hr later one of the groups was injected intraperitoneally with 1 ml NBCS. At given time points the labeling index and the total numbers of both peripheral blood monocytes and peritoneal macrophages were determined, and from these values the total numbers of labeled peripheral blood monocytes and peritoneal macrophages were calculated (Figs. 7 and 8). The results were compared with the data obtained in an earlier study done in normal mice and in mice with an acute inflammatory response induced by newborn calf serum published elsewhere.

An inflammatory exudate in animals treated with azathioprine shows a pat-
Fig. 7. The effect of long-term treatment with 3 mg/kg azathioprine (×) on the number of labeled peritoneal macrophages in normal mice and mice in which an acute peritoneal inflammation was provoked with NBCS (+), 1 hr after an intravenous 3H-thymidine injection (•). For comparison, the course of labeled peritoneal macrophages in similar mice not treated with azathioprine is given.

Fig. 8. The effect of long-term treatment with 3 mg/kg azathioprine (×) on the number of labeled peripheral blood monocytes of normal mice and mice in which an acute peritoneal inflammation was provoked with NBCS (+), 1 hr after an intravenous 3H-thymidine injection (•). For comparison, the course of labeled monocytes in similar mice not treated with azathioprine is given.
tern quite different from the one seen in the control animals: at 24 hr the number of labeled peritoneal macrophages is 25% lower and at 96 hr 65% lower than in the untreated animals (Fig. 7). Animals treated with azathioprine but not with NBCS have fewer labeled peritoneal macrophages than normal animals during the first 48 hr after labeling.

The labeling of monocytes in azathioprine-treated animals shows roughly the same pattern as the control animals during the first 12 hr of the inflammatory response. After that there is a leveling off: at 48 hr the peripheral blood contains 113 labeled monocytes per cu mm, which is about 25% of the maximum value found for the control animals at that time point (Fig. 8). There is also a great discrepancy between the number of labeled monocytes in azathioprine-treated and normal animals; the level in the former remains considerably lower at all time points (Fig. 8).

**DISCUSSION**

The findings obtained in the present study show that azathioprine causes a reduction in the number of mononuclear phagocytes in the circulation and peritoneal cavity. Since the macrophages in the tissues are not a static population but are continuously replaced by monocytes from the peripheral blood, the action of a drug affecting the number of tissue macrophages can only be interpreted properly by taking into consideration its effect on the peripheral blood monocytes.

In the peripheral blood, azathioprine reduces the number of monocytes; the extent depends on the dose and duration of administration. During the first 48 hr of treatment with a high dose (200 mg/kg) of azathioprine, the reduction in the number of peripheral blood monocytes amounts to $3.1 \times 10^5$ cells per mouse and is doubled in the next 48 hr. If the rapid disappearance between the 48th and 96th hr is regarded exponentially, the monocytes leave the circulation with a half-time of 17 hr, which closely approaches the half-time (22 hr) found for peripheral blood monocytes in normal mice. This would indicate that during that period no newly formed monocytes appear in the circulation, a conclusion which is supported by labeling studies showing that, in contrast to the situation in normal animals, there is no increase in the labeling index of peripheral blood monocytes after 36 hr of high-dosage azathioprine treatment.

In the bone marrow, the number of monocytes decreased by about 60% during 96 hr treatment with the high dose of azathioprine, and labeling studies demonstrated that the production of monocytes in the bone marrow was decreased to about 10%. Analysis of the effect of this drug on the mitotic activity of the monocyte precursors, the promonocytes, showed that azathioprine inhibited the mitotic activity of these cells by prolonging the DNA-synthesis phase. These studies thus proved that the monocytopenia in the peripheral blood caused by azathioprine was due to the reduced monocyte production.

The effect of azathioprine appears to be reversible, because, 24-48 hr after treatment is stopped (depending on whether 3 or 200 mg/kg azathioprine is given), the number of monocytes starts to increase again.

During an acute inflammatory reaction in the tissues, monocytosis usually occurs, with a maximum of 2.25-3.5 times the number of monocytes found in
normal animals. This effect, too, is inhibited by azathioprine, the extent of the depression being dependent on the dose administered. During treatment with a low dose of azathioprine, a limited increase in the number of peripheral blood monocytes occurs only during the first 12 hr of the inflammatory response, but during treatment with a high dose of the drug the monocyte response is very small. This effect is due to the absence of an increase in monocyte production, which otherwise occurs in animals with an acute inflammatory reaction not treated with azathioprine.

With respect to the effect of azathioprine on the peritoneal macrophages, the number of these cells in normal animals is affected by a high dose of azathioprine given for more than 4 days, after which the number of macrophages shows a marked decline. Labeling studies have demonstrated that during azathioprine treatment, the influx of monocytes from the circulation into the peritoneal cavity is diminished or completely abolished, depending on the dose administered. This reduced replacement of peritoneal macrophages is caused by the monocytopenia in the circulation, as no cells are available to migrate into the peritoneal cavity. (Control studies with azathioprine or 6-mercaptopurine in the culture medium in concentrations of 0.3 to 30 \( \mu g/ml \) show no effect on the viability of peritoneal macrophages incubated for 24 hr as determined by trypan blue exclusion and the phase-contrast picture.) During treatment with a low dose of azathioprine, the number of peritoneal macrophages is only slightly reduced because sufficient numbers of monocytes are still present in the circulation to replace the slow loss of macrophages from the peritoneal cavity.

While the influx of peripheral blood monocytes into the peritoneal cavity is absent, the decreases in the number of peritoneal macrophages, i.e., 0.56 \( \times 10^4 \) cells per hr, provide an estimate of the minimal turnover time of the peritoneal macrophages (total number of peritoneal macrophages divided by the reduction per hour), which is about 19 days. A similar calculation based on the results obtained after 7 days of treatment with hydrocortisone acetate gives a peritoneal macrophage turnover time of about 27 days.

Azathioprine also affects the acute inflammatory response in the peritoneal cavity. A high dose gives almost complete suppression of the influx of monocytes into the site of the lesion, whereas during treatment with a low dose (3 \( mg/kg \)) this influx remains at 75\% (3.0 \( \times 10^5 \) mononuclear phagocytes) of the "normal" inflammatory response during the first 24 hr but then ceases completely, in contrast to the pattern occurring in the inflammatory exudate induced in normal mice. Because of this cessation, at 96 hr the inflammatory exudate contains only 57\% of the number of macrophages found in normal animals with an inflammatory reaction. The smaller number of mononuclear phagocytes in the inflammatory exudate can be explained by the absence of a sufficient number of monocytes in the circulation to migrate into the lesion.

The results of the present quantitative study on the effect of azathioprine on an inflammatory response in mice are compatible with the findings for treatment with 6-mercaptopurine in other animal species. Hurd and Ziff observed a significant correlation in rabbits between the mononuclear cells (not further characterized as mononuclear phagocytes or lymphocytes) in an inflam-
matory skin lesion and the number of circulating monocytes. Phillips and Zweiman found in the guinea pig that 6-mercaptopurine causes a depression of the peripheral blood monocyte level and a lowered number of macrophages in an inflammatory exudate in the peritoneal cavity.

A question remains whether or not results obtained in animal models can be applied to man. It is reasonable to assume that the mechanism of drug action is alike in man and animals, but the dose-effect relationship could be quite different. For example, the immunosuppressive activity of azathioprine is about 20 times lower in mice than in man, and the maximum tolerated dose for mice is about 65 times higher (i.e., 200 mg/kg/day) when the dose is expressed per kilogram body weight. However, when the dosage of drug is based on body surface, the maximum tolerated dose for mice and man differs only by a factor of 6 (calculated with the conversion factor given by Freireich). When these differences are taken into consideration, the low dose (3 mg/kg/day) applied in this study, which caused a marked monocytopenia and suppression of the inflammatory response in mice, is about equivalent to a nontoxic, immunosuppressive, anti-inflammatory dose used in man.

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