STUDIES ON THE ANTI-INFLAMMATORY ACTION OF 6-MERCAPTOPURINE*

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Within recent years, there has been considerable interest in the use of antimetabolic drugs in organ transplantation and for the treatment of various immunologically related diseases. These drugs have been shown to have both immunosuppressive (1-3) and anti-inflammatory effects (4-6), but the actual mechanisms underlying these effects are not well established. 6-Mercaptopurine (6-MP), which has an inhibitory effect on nucleic acid synthesis (7-10), is one of these agents which has been intensively studied. In 1962, Page, Condie, and Good (4) demonstrated that 6-MP virtually eliminated the appearance of mononuclear cells at a local inflammatory site. This effect developed only after a requisite period of administration of the drug, the duration of which was inversely related to the dose. The same anti-inflammatory effect was subsequently demonstrated in humans who were receiving 6-MP for the treatment of neoplastic disease (5).

The mononuclear cell populations of the tuberculin hypersensitivity lesion (11), the inflammatory reaction produced by local injection of fibrinogen (12) and of peritoneal exudates (13) have been shown to consist largely of hematogenous cells thought to be derived from rapidly proliferating precursors in the bone marrow. In the present report, the possibility was investigated that 6-MP might suppress inflammation through interference with the proliferation of the precursors of these mononuclear cells. In these experiments, the effect of 6-MP on the intensity of mononuclear cell infiltration of an induced skin lesion in rabbits has been correlated with the numbers of the various types of mononuclear cells in the blood. The replication of these cells in vitro, as measured by incorporation of tritiated thymidine (^{3}H-Tdr), has been determined simultaneously. The results obtained have demonstrated that administration of 6-MP decreases predominantly the numbers of monocytes and large, proliferating

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lymphocytes in the blood concurrently with the decrease of mononuclear cells in inflammatory sites. This action of 6-MP appears to be the basis of its anti-inflammatory effect.

Materials and Methods

Animals.—In all experiments, albino rabbits of both sexes, weighing 2.0–3.0 kg, were obtained from a single local breeder in groups of eight from the same litter and randomly separated into two equal groups. One was treated with 6-MP and the other served as a control group. Both groups were pair-fed a standard Purina pellet diet in order to keep weight gain or loss equal in the two groups.

6-Mercaptopurine.—6-MP was obtained in the form of the sodium salt in 0.5 g sterile vials (kindly supplied by Dr. Donald S. Searle of Burroughs Wellcome & Co., Tuckahoe, N. Y.) and water added to a concentration of 37.5 mg/ml. A fresh vial was used daily, and the dissolved drug injected immediately after addition of water. Injections were given intravenously into the marginal ear vein daily for 10 days in a dosage of 18 mg/kg. Injected solutions had a pH of 10.5. They were only mildly irritating to the vein. Control animals received equivalent volumes of pH 10.5 phosphate-buffered saline.

Turpentine Injections.—In order to increase the blood levels of large mononuclear cells, turpentine was injected subcutaneously on day 8. 0.1 ml of a solution of turpentine in olive oil (1:5) was injected into four sites on the back.

Inflammatory Response.—The studies of Page and coworkers (4) were repeated using four 6-MP treated animals and four controls in each experiment. On day 9, 0.1 ml of fresh egg white, mixed with India ink, was injected subcutaneously into four sites along the rabbit's shaved back on the opposite side from the turpentine injections. Animals were anesthetized with intravenous Somnopentyl (pentobarbital sodium, Pitman-Moore, Indianapolis, Ind.) and biopsy specimens obtained 4–6 hr after injection. The loose subcutaneous connective tissue was spread on clean glass slides, the tissue fixed by air-drying, and then stained with Wright-Giemsa stain as described by Page and coworkers and by Kolouch (4, 14). Counts of 500 cells were done on the tissue sections, differentiating only between mononuclear and polymorphonuclear cells. Percentages of mononuclear cells containing carbon particles derived from India ink were also tabulated.

Cell Counts.—Total white blood cell and differential counts were done on blood obtained from the ear in treated and control animals on days 9 and 11. In the differential counts, a total of 500 cells were counted per animal. Leukocytes were classified as neutrophils (including eosinophils), basophils, monocytes, and large, medium, and small lymphocytes. Small lymphocytes were 7 μ or less in diameter. Medium lymphocytes ranged between 7–14 μ and large lymphocytes were larger than 14 μ in diameter.

Monocytes were classified according to the criteria of Spector and coworkers (12) for "typical" monocytes. In order to better define this cell type, differential counts were carried out on buffy coat preparations simultaneously with measurements of phagocytosis of carbon particles in vitro. To 40 ml of heparinized blood, 10 ml of 1% dextran and 12 drops of a 1:50 dilution of carbon black (Gunther Wagner-Pelikan, Hanover, Germany, No. C11143a) were added, and the blood was allowed to stand for 1 hr at 37°C. The sedimented erythrocytes were then separated, and three drops of the supernatant were placed in the well of a Shandon cytocentrifuge (SCA-0001) and spun at 400 rpm. Smears, made from these preparations, were then stained with Wright's stain and differential counts performed on 500 cells. Percentages of the mononuclear cells which contained carbon were tabulated. In four untreated and four treated animals examined on day 13, mean values of 86% and 65%, respectively, of the

cells with the appearance of monocytes had phagocytosed carbon particles, while this was rare in the case of the other mononuclear cells.

^{3}H-Tdr Incorporation.—The method of Vischer and Stastny (15) was used. Supernatant plasma, obtained as above, following dextran sedimentation was centrifuged at 2000 rpm and the cell pellet was resuspended in Hanks' minimum essential medium (Grand Island Biological Co., Grand Island, N.Y.) with added glutamine, penicillin, streptomycin, and Mycostatin. Cell counts were adjusted to a concentration of 10^7 cells/ml. 1 ml was placed in each of four plastic flasks and to each flask was added 3.25 ml of the medium, 0.75 cc of plasma from the same animal, and 0.1 ml ^{3}H-Tdr containing 0.1 μc (specific activity, 0.67 c/mM, New England Nuclear Corp., Boston, Mass.). In one flask, the ^{3}H-Tdr was added after the culture period, and this served as a zero time control. The other three flasks were treated identically. After incubation for 1 hr in a 37° incubator, the contents were transferred to test tubes, centrifuged, and washed five times with phosphate-buffered saline, pH 7.2. Two of these washings contained an excess of cold thymidine as described by Holm and Perlmann (16). The cells were then dissolved in 1 ml of 0.1 N NaOH, transferred to bottles containing Bray's phosphor, and counted in a Beckman 200 liquid scintillation counter with thixotropic gel (CAB-O-SIL, Packard Instrument Co., Downers Grove, Ill.) Counts were corrected for quenching and efficiency, and results expressed as disintegrations per minute (dpm).

Radioautography.—10^7 cells were obtained and pulse labeled for 1 hr in vitro as described above, then removed from the flask, centrifuged, suspended in fetal calf serum, and centrifuged again. The moist pellet was brushed on clean glass slides which were then dipped in Kodak NTB-3 nuclear track emulsion, stored 1 wk in the cold, and developed. Slides were then stained with Leishman's stain and differential cell counts made. A minimum of 5 grains per nucleus was required to record a cell as labeled.

Statistical Methods.—A student's t test for comparison of two independent samples was used to compare data from treated and control animals with the assumption that the alternate hypothesis was one sided. For the correlation of numbers of blood mononuclear cells with tissue mononuclear cells expressed as per cent of total cells, a computer program was used to compute means, standard deviations and Pearson correlation coefficients. An IBM 1800 digital computer was used for all calculations.

RESULTS

Effect on Circulating Leukocytes.—On the 9th day of 6-MP administration and following injection of turpentine subcutaneously on day 8, no difference in total blood leukocyte counts was observed in treated and control animals (Table I). Total numbers of neutrophils, total mononuclear cells, and medium and small lymphocytes (Tables I and II) were all similar in both groups at this time. There were, however, significant decreases in the large lymphocytes and monocytes in the treated animals relative to the controls.

Following subcutaneous injection of egg white and India ink on the 9th day and excision of the injection site shortly thereafter, the above counts were repeated on day 11. On this day, the reductions in the numbers of monocytes and large lymphocytes in the treated animals had become marked and highly significant. Moderate, but statistically significant decreases in counts of total leukocytes, neutrophils, total mononuclear cells (Table I), and medium-sized lymphocytes (Table II) were now observed in the treated animals as com-

pared with the controls. On the other hand, again no difference was seen in the small lymphocyte counts.

It may be noted (Table II) that the marked differences in the monocyte and large lymphocyte counts of the treated and control animals seen on day 11 reflect, to a considerable extent, increases in the numbers of these cell types which occurred in the control group between the 9th and 11th days. These increases were, presumably, a result of the inflammatory stimulus produced by the turpentine and egg white injections and the biopsy procedure. The differences noted, therefore, appear to reflect an inhibitory effect of 6-MP on increases in large lymphocytes and monocytes which occurred in the controls as a result of the inflammatory stimuli.

TABLE I

Blood Leukocyte Counts in 6-MP-Treated and Control Animals

Day	Group	Total leukocytes	Neutrophils	Total mononuclear cells
9	Controls (12)	10,108 (4100–18,300)	4052 (1435–8630)	6055 (2655–9670)
	6-MP (12)	9191 (4750–15,000)	3852 (1790–6750)	5339 (2718–10,200)
		*(N.S.)**	*(N.S.)*	*(N.S.)*
11	Controls (10)	16,375 (6750–28,250)	5408 (2216–9100)	10,967 (4534–19,150)
	6-MP (10)	9670 (3700–17,750)	3232 (889–6830)	6438 (2811–12,140)
		($P < 0.025$)	*($P < 0.025$)*	*($P < 0.025$)*

* *N.S.*, not significant.

Effect of 6-MP on Tissue Lesion.—When a mixture of egg white and India ink were injected subcutaneously after 8 days of 6-MP administration and biopsy specimens of subcutaneous tissue taken 4–6 hr later using the technique of Kolouch (14), the results of Page and coworkers (4) were confirmed, i.e., a significant decrease in the mononuclear cell infiltrate in the areas of induced inflammation was noted. Per cent of mononuclear cells in the tissue lesions fell from 32% in the controls to 9.6% in the 6-MP–treated animals (Table III, Figs. 1 and 2). Although, as noted above, both the large lymphocyte and monocyte counts in the blood were significantly depressed in the 6-MP–treated group at this time, a significant correlation was found (Table IV) only between

blood monocyte counts and percent tissue mononuclear cells in the 6-MP–treated animals. This correlation was highly significant. There was no correlation with any other blood mononuclear cell type.

TABLE II

Blood Mononuclear Cell Counts in 6-MP–Treated and Control Animals

Day	Group	Small lymphocytes	Medium lymphocytes	Large lymphocytes	Monocytes
9	Controls (12)	1158 (107–2949)	4056 (1078–7580)	444 (211–770)	268 (56–660)
	6-MP (12)	1225 (196–3786)	3668 (377–8840)	185 (14–460)	99 (0–418)
		(*N.S.*)	(*N.S.*)	($P < 0.002$)	($P < 0.025$)
11	Controls (10)	1136 (268–3160)	7975 (3454–12,920)	1038 (216–2145)	522 (108–1300)
	6-MP (10)	1314 (443–2550)	4665 (673–10,260)	230 (36–612)	90 (0–195)
		(*N.S.*)	($P < 0.05$)	($P < 0.001$)	($P < 0.005$)

TABLE III

Simultaneous Cell Counts in Blood and Tissue Lesion on Day 9

Group	Total mononuclear cells in blood	Large lymphocytse in blood	Monocytes in blood	Mononuclear cells in tissue lesion
				%
Control (12)	6055	444	268	32.1 (3.2–51.0)
6-MP (12)	5339	185	99	9.6 (0.8–45.8)
	(*N.S.*)	($P < 0.002$)	($P < 0.025$)	($P < 0.002$)

The morphology of the mononuclear cells in the inflammatory skin lesion, using the Kolouch technique, was not adequate for accurate differential counts of the mononuclear cells in most cases. However, typical blood monocytes and lymphocytes of varying sizes were seen. Phagocytosed carbon particles were observed in mononuclear cells in all of 24 preparations. The percentage of mononuclear cells labeled with carbon varied between 25% and 75% in the

treated animals (mean, 50%) and 10% and 97% in the controls (mean, 54%). There appeared to be no difference between the two groups in this respect. Thus, a large fraction of the mononuclear cells present in the inflammatory lesion in both groups showed phagocytic activity.

TABLE IV

Correlation between Number of Mononuclear Cells in the Blood and Per Cent Mononuclear Cells in Tissue Lesions in 6-MP-Treated Animals

Cell type in blood	Correlation with per cent tissue mononuclear cells (correlation matrix)
Small lymphocyte	−0.218 (*N.S.*)
Medium lymphocyte	0.064 (*N.S.*)
Large lymphocyte	0.121 (*N.S.*)
Monocyte	0.750 ($P < 0.005$)

TABLE V

Tritiated Thymidine Incorporation of Blood Mononuclear Cells in Treated and Control Animals (dpm $^3HTdr/10^7$ Mononuclear Cells)

	mononuclear cells, dpm per 10^7	
	Day 9*	Day 11‡
Controls	12,052 (6880–18,000)	7822 (3290–12,580)
6-MP-treated	4725 (3120–8580)	4593 (1245–7750)
Per cent of control	39.2 ($P < 0.025$)	58.5 ($P < 0.05$)

*12 animals in each group.
‡10 animals in each group.

Incorporation of ^3H-Tdr by Mononuclear Cells of the Blood.—The radioactive counts (dpm) incorporated by the blood mononuclear cells of the 6-MP-treated animals were 39.2% of the control value on day 9 and 58.5% on day 11 (Table V). It is of interest that although there was a greater number of large mononuclear cells in the blood of the control animals on the 11th day (Table II) than on the 9th day, the radioactive counts per 10^7 mononuclear cells

actually fell, suggesting that there was a smaller proportion of dividing cells in the peripheral blood at this time.

Radioautographic Experiments.—The percentage of each cell type labeled during a 1 hr in vitro incubation of the blood mononuclear cells with ^{3}H-Tdr was measured by radioautography. In the control animals, on day 9 (Table VI), 45.6% of the large lymphocytes and 6.3% of the medium lymphocytes were labeled during the 1-hr period. There was little labeling of the other cell

TABLE VI

Distribution of ^{3}HTdr Label in 2000 Mononuclear Cells of Untreated Animals Following 1-hr Incubation Period

Cell type labeled	No. cells counted	No. with label	Per cent with label
Large lymphocyte	46	21	45.6
Medium lymphocyte	290	12	6.3
Small lymphocyte	1758	2	0.1
Monocyte	6	0	0

TABLE VII

Effect of 6-MP on In Vitro ^{3}HTdr-Labeling of Mononuclear Cells on Day 9

Cell type labeled	No. of cells/10^6 mononuclear cells with label	
	Control (5)	6-MP (7)
Large lymphocyte	10,598	2135 ($P < 0.05$)
Medium lymphocyte	5840	1780 ($P < 0.025$)
Small lymphocyte	1061	0 (*N.S.*)
Monocyte	0	356 (*N.S.*)
Total.....	17,499	4271

types. The values on day 11 were similar to those on day 9. Thus, the predominant mononuclear cell type which was labeled in vitro appeared to be the large lymphocyte. An example of this type of cell is shown in Fig. 3.

When the number of mononuclear cells of each type which were labeled was determined (Table VII), it was observed that the principal cell types contributing to the pool of labeled cells were large and medium lymphocytes, although the contribution of the large lymphocytes was clearly greater. This cell type, which formed only 7.3% of the total mononuclear cells, constituted 60.4% of the labeled cells. In the 6-MP–treated animals, there was a marked reduction in labeled cells, i.e. from 17,500 per 10^6 mononuclear cells in the controls to 4270 per 10^6 mononuclear cells, a fall of 76%. Thus, the radioautographic

measurements confirm the scintillation counting data presented above in the finding that 6-MP decreased the numbers of cells capable of in vitro replication, and provide the additional information that the cell type chiefly involved in this in vitro change is the large lymphocyte.

DISCUSSION

Evidence that 6-MP may decrease the infiltration of mononuclear cells in an inflammatory lesion induced in the rabbit by egg albumin was presented by Page, Condie, and Good (4). A similar phenomenon has been described using a skin window technique in humans receiving 6-MP and other antimetabolites (5). The possibility that 6-MP might bring about this effect by inhibiting the proliferation of the precursors of the mononuclear cells which accumulate in inflammatory lesions was investigated in the present study.

The mononuclear cells present in inflammatory lesions (12, 17) and peritoneal exudates (13) are short-lived cells which are probably derived from rapidly proliferating precursors in the bone marrow (12, 17). Kosunen and coworkers (11), examining delayed tuberculin reactions, described the mononuclear cells in these lesions as "medium-large" lymphocytes which became histiocytes in time. Spector, Walters, and Willoughby (12), studying a fibrinogen-induced inflammatory skin lesion in rats injected with ^{3}H-Tdr and carbon, observed that the percentage of tissue mononuclear cells which was doubly labeled was the same as the per cent of doubly labeled monocytes in the peripheral blood. They concluded from this that the predominant cell in the inflammatory lesion was the blood monocyte.

Since 6-MP affects both DNA and RNA synthesis (18, 19), it would be expected to exert a major influence on the mitosis of rapidly replicating cells. This is consistent with the fact that this drug exerts its most profound toxic effects on rapidly proliferating tissues, e.g. bone marrow, skin, and intestinal epithelium (20, 21). As a result of its action on the bone marrow, one would expect a decrease in the blood levels not only of polymorphonuclear leukocytes but also of monocytes and medium to large lymphocytes, since these cell types are known to be rapidly labeled in the bone marrow and blood following a single injection of ^{3}H-Tdr in vivo (11, 13, 22). Latta and Gentry (23) have, in fact, observed marked decreases in the counts of large lymphocytes and monocytes in mice treated with 6-MP, and Miller and Cole (24, 25) have found that the only resistant cells in lymph nodes of rats treated for long periods of time with 6-MP and other anti-proliferative agents were small lymphocytes and plasma cells.

In the present experiments, rabbits have been injected with 6-MP and the effects of this agent have been examined concurrently on the counts of the various mononuclear cell types in the blood and the per cent of mononuclear cells in a skin lesion produced by local injection of egg albumin. Following administration of 6-MP, there was, as previously reported (4), a marked decrease in the number of mononuclear cells in the skin lesion. This was paralleled by a fall in the large mononuclear cells present in the blood. On a dosage of 18 mg per kg of 6-MP per day, the first discernible effects were noted on day 9, at which time there were significant decreases in circulating large lymphocytes and monocytes. The counts of total leukocytes, neutrophils, and total

mononuclear cells in the blood were essentially unchanged at this time. By day 11, however, when the circulating large lymphocytes and monocytes had fallen to very low levels, smaller, but significant decreases in the counts of neutrophils, total mononuclear cells, and medium lymphocytes had occurred. The numbers of small lymphocytes, nevertheless, continued to remain unchanged. In fact, although not here reported, small lymphocytes became the predominant white blood cell type in the peripheral blood following administration of 6-MP at the same dosage level for a period of 4 wk.

From the above observations, it would appear that the reduction in mononuclear cell infiltration of the skin in the 6-MP–treated animals was paralleled most closely by decreases in large lymphocytes and monocytes in the blood. However, when the counts of the various mononuclear cells in the blood of these animals were correlated with per cent of mononuclear cells in the skin lesion, a significant correlation was found only in the case of the blood monocyte. This is in agreement with the conclusion of Spector and coworkers (12) that the monocyte is the principal blood mononuclear cell which participates in inflammatory skin lesions. It suggests that the large lymphocyte does not penetrate inflammatory skin lesions as readily as the monocyte, at least in the 4–6 hr period following injection of egg albumin.

An increase in total blood mononuclear leukocytes was observed in the control animals between days 9 and 11. This resulted presumably from the stimulus produced by the preceding turpentine and egg white injections and by the skin biopsy. It is likely also that the counts on day 9, particularly of large lymphocytes and monocytes, were already raised by the turpentine injection which had been injected on day 8. This agent was given in order to provide an inflammatory stimulus which would effect the release of adequate numbers of large mononuclear cells into the blood as found by Spector and coworkers using fibrinogen (12). Thus, it would appear that the lower cell counts observed in the 6-MP–treated animals resulted, in all likelihood on day 9, and clearly on day 11, from an inhibition imposed by 6-MP on increases in these counts generated in the control animals by inflammatory stimuli.

The polymorphonuclear leukocytes of the blood did not decrease on 6-MP until day 11, and even at this time the fall to 59.7% of the control values, although significant, was much smaller than the decreases in monocytes and large lymphocytes which fell to 17.2% and 22.2% of control values respectively. Presumably, this lag reflects the time required for maturation of the neutrophil and its subsequent storage period in the bone marrow. It has been shown by Otteson and Hamilton (26, 27) that following injection of ^{14}C in man, labeling of the DNA of lymphocytes precedes by several days the labeling of the DNA of neutrophils, the latter being delayed in their appearance in the blood from 4 to 6 days. If, as the data suggests, the effect of 6-MP is on proliferating precursor cells, one would expect from the present findings that the more rapidly labeled cell populations of the blood, i.e., the bone marrow derived mononuclear cells would be depleted earlier than the neutrophils.

Evidence that proliferating cells are decreased in number in the blood of 6-MP–treated animals was also obtained in experiments in which in vitro–incorporation of ^{3}H-Tdr into blood mononuclear cells was measured by scintillation counting and radioautography. The reduced incorporation of ^{3}H-Tdr observed in the cells of the 6-MP–treated animals on day 9, indicated a diminution in the number of proliferating cells in the blood. Presumably, the greater incorporation observed in the controls on day 9 as compared with day 11, reflected labeling of increased numbers of proliferating

cells liberated into the blood as a result of the stimulus produced by the prior injection of turpentine. By day 11, the amount of in vitro labeling was smaller, presumably, because the numbers of proliferating cells had already decreased during the 2-day interval following the inflammatory stimuli. The occurrence of increased peripheral mononuclear cell counts, noted on day 11, is not inconsistent with a decrease in proliferating cells, since short-lived mononuclear cells may not continue to manifest the capacity for in vitro cell division 48 hr after the inflammatory stimulus responsible for their release.

Radioautography on day 9 showed that the large lymphocyte had the highest percentage of labeling when cultured in vitro with ^{3}H-Tdr. A relatively smaller percentage of medium lymphocytes and a negligible proportion of small lymphocytes and monocytes were labeled. When the total numbers of cells of each cell type with label were determined, the large and medium lymphocytes were the most numerous labeled cells. In the 6-MP–treated animals, the numbers of these cells bearing label were significantly reduced.

The preceding discussion has dealt primarily with an inflammatory lesion caused by egg albumin, but it is reasonable to expect that the same considerations would apply to the delayed hypersensitivity reaction. Lubaroff and Waksman (17) injected bone marrow cells into irradiated animals and subsequently induced a delayed tuberculin hypersensitivity lesion. The majority of mononuclear cells in this lesion were of bone marrow origin. From this observation, one would expect that immunologically induced mononuclear cell infiltration would also be diminished by 6-MP. Borel and Schwartz (6) have, in fact, demonstrated that this drug suppresses delayed hypersensitivity without inhibiting humoral antibody production.

As indicated above, the majority of mononuclear cells which accumulate in inflammatory lesions are large mononuclear cells, i.e., monocytes (12) and large lymphocytes (11). In the present experiments, a high correlation was observed between the levels of monocytes in the blood and the per cent mononuclear cells in the tissue lesion induced by egg albumin. Furthermore, a mean of 52% of the tissue mononuclear cells, and in some lesions as high as 97%, phagocytosed carbon. These observations suggest that the major mononuclear cell type in the tissue lesion is the monocyte, although the possibility that the large lymphocyte also accumulates in the tissue is not ruled out by the present results. Since 6-MP appears to reduce inflammation by inhibiting proliferation of precursor cells of the large mononuclear cells of the blood, its action on precursors of the monocyte would, presumably, be its most important effect in the present experiments. However, available evidence (11) indicates that, in the case of the tuberculin reaction, the large lymphocyte also makes an important contribution. The effects of inhibition of proliferation of precursors of these cell types would, of course, become most apparent in the presence of an active inflammatory reaction, since proliferation of precursors in sites such as the bone marrow would be most active at this time.

Current concepts of the mechanism of action of anti-metabolic drugs in the treatment of immunologically related diseases and in the preservation of renal homografts generally accept their action as being primarily immunosuppressive (1–3). However, it is not certain to what extent the beneficial effects obtained result from suppression of the immune response and to what degree they might result from an anti-inflammatory effect. Swanson and Schwartz (1) found that clinical improvement in patients

with "immunological" disease was not paralleled by suppression of antibody formation when such patients were immunized with hemocyanin and treated with azathioprine. This observation raised the possibility that the clinical improvement observed in these patients resulted from an anti-inflammatory effect. The manifestation by thiopurines of a predominantly anti-inflammatory effect appears likely in patients with immunologically mediated disease who are benefited by these drugs, since the immune response is presumably already well established in these patients and since available evidence (25, 28, 29) indicates that 6-MP does not exert a significant immunosuppressive effect on an established immune response.

The immunosuppressive action of 6-MP is greatest at the time of induction of the primary antibody response (2, 3, 25, 28, 29, 32, 33). While the macrophage and certain short-lived lymphocytes appear to play important roles in the induction phase of the immune response (34–36), long-lived mononuclear cells are believed to be mainly concerned with antibody synthesis and the conservation of immunological memory (30, 31). Miller and Cole have, in fact, demonstrated that long-lived small lymphocytes and plasma cells are resistant to prolonged treatment with 6-MP and other immunosuppressive agents (24, 25). Thus, with regard to antibody formation, the action of 6-MP appears to be manifested most effectively in relation to a phase of cellular proliferation which is prominent at the time of induction. In the use of azathioprine or 6-MP for the suppression of homograft rejection, the immune response to the homograft may well be suppressed when treatment with these drugs is begun at the time of transplantation, but following one or more episodes of rejection, when immunity has presumably become established, it is likely that the anti-inflammatory effect of 6-MP assumes an important role in maintaining the graft.

SUMMARY

The mechanism of action of 6-mercaptopurine (6-MP) on an egg albumin–induced inflammatory lesion in the skin has been studied in rabbits treated with 6-MP in a daily dosage of 18 mg/kg. Relative to control animals, significant decreases in the numbers of large lymphocytes and monocytes in the blood were observed in the 6-MP–treated animals by the 9th day of treatment, without significant decrease in the numbers of polymorphonuclear leukocytes and small and medium lymphocytes. Concurrently, a significant decrease was also seen in the percentage of tissue mononuclear cells in the inflammatory skin lesion. There was a highly significant correlation between the numbers of monocytes in the blood and the per cent of mononuclear cells in the lesion. A mean of 52% of the mononuclear cells in the tissue lesion phagocytosed carbon offering further evidence that the major cell involved was the blood monocyte.

In vitro incorporation of ^{3}H-Tdr by blood mononuclear cells was significantly reduced in the 6-MP–treated animals as determined by scintillation counting and radioautography. The large lymphocyte was the predominant cell type which was labeled in vitro. Small lymphocytes and monocytes were rarely labeled.

The data obtained suggest that the anti-inflammatory effect of 6-MP, reflected in these experiments by a decrease in mononuclear cells in a tissue lesion,

results from suppression of a bone marrow response to local inflammation, affecting principally proliferating precursors of blood monocytes and large lymphocytes. The possible importance of this action of 6-MP in the treatment of inflammatory and immunologically mediated disease is discussed.

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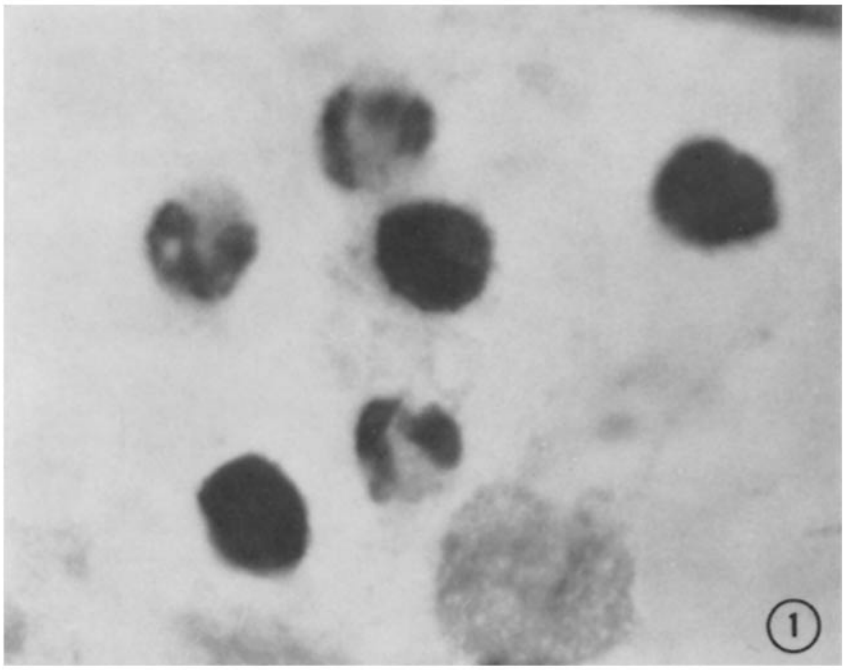
FIG. 1. Appearance of subcutaneous lesion 4–6 hr after injection of egg white and India ink in normal rabbit. Both polymorphonuclear and mononuclear leukocytes are seen × 1000.

FIG. 2. Appearance of subcutaneous lesion 4–6 hr after injection of egg white and India ink in rabbit following treatment with 6-MP (18 mg/kg) for 8 days. Adequate numbers of polymorphonuclear leukocytes are seen, but no mononuclear cells are present × 1000.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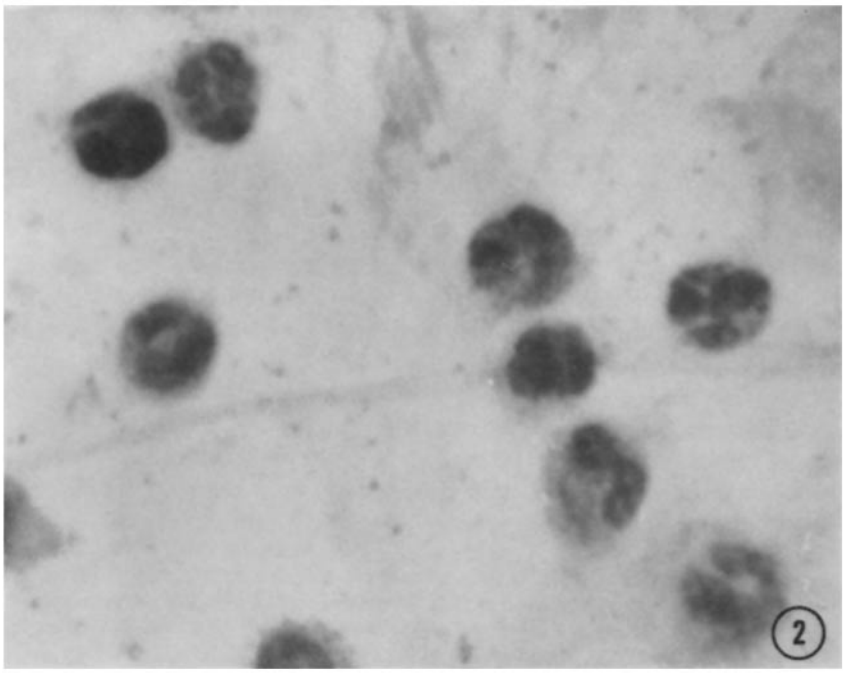
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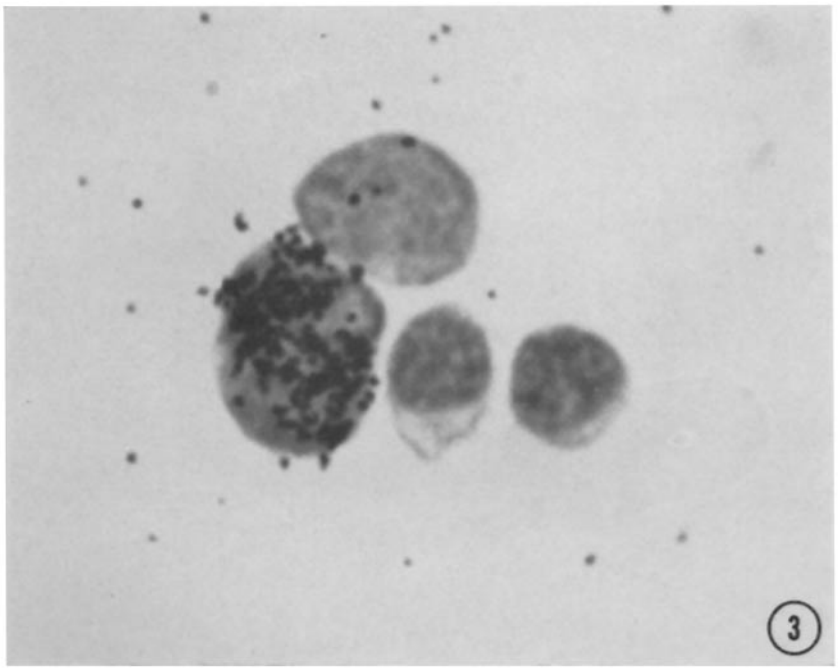

FIG. 3. Radioautograph in a control animal showing labeling of a large lymphocyte, incubated with ^{3}H-Tdr in vitro for 1 hr. A nonlabeled medium to large lymphocyte and two small lymphocytes are also present × 1000.