THE EFFECT OF 6-MERCAPTOPURINE ON DELAYED HYPERSENSITIVITY IN GUINEA PIGS*

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The relationship between delayed hypersensitivity and antibody production has been intensively investigated. Included in the attempts to define the relationship between the two immune phenomena has been the use of antimetabolites. A folic acid antagonist, methotrexate, suppresses both antibody formation and delayed hypersensitivity (1). In contrast, a nucleic acid antagonist, 6-mercaptopurine (6-MP), suppresses antibody formation in rabbits (2), but has been reported to have no effect on delayed hypersensitivity in guinea pigs (3). The following study was initiated to determine whether this difference in response to 6-MP adequately differentiates the two immunologic phenomena and to consider the possibility that the effect of 6-MP is species-specific, a suggestion made in a recent paper which reported that 6-MP treatment did not affect either antibody formation or delayed hypersensitivity in the guinea pig (4). This paper describes experiments in which treatment with 6-MP suppressed the development of delayed hypersensitivity in guinea pigs during the period of drug administration.

Materials and Methods

Animals.—Guinea pigs were Hartley strain albinos of both sexes and weighed between 400 and 550 gm. These animals were weighed daily.

Sensitization.—Animals were sensitized by intraperitoneal injection of 0.1 ml of a 10 per cent suspension of living BCG in physiologic saline (1.0 ml of packed BCG in 10 ml saline). Phipps' strain BCG was harvested in saline after growth on Petragnani's medium.

6-Mercaptopurine[1].—6-MP solutions were prepared daily by dissolving the drug in 1 N

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[1] Purinethol brand 6-MP was generously supplied by Donald S. Searle, M.D., Medical Director, Burroughs, Wellcome and Co., Inc., Tuckahoe, New York.

NaOH (0.15 gm/ml) at 37°C. Injections of this mildly irritating solution (pH of 10) were intraperitoneal (ip) or intramuscular (im) into the deep muscles of the back.

Cutaneous testing.—Animals were tested intradermally on the flank with 0.005 mg PPD (Parke, Davis & Company) in 0.1 ml (equivalent to 1:100 OT). Reactions were observed and areas of induration were measured at 4, 8, 24, and 48 hours. Measurements reported are the maximum responses, which in all cases were observed at 24 hours.

Fasting.—The fasted animals were not fed after stimulation with BCG but given only water *ad lib.* supplemented with vitamin C at a concentration of 250 mg/liter.

TABLE I

Skin Reactions of Guinea Pigs Tested with PPD for Cutaneous Hypersensitivity

Treatment	Fraction of animals tested having positive reactions*	
	Day 10 after BCG infection	Day 19 after BCG infection
6-MP, 50 mg/kg/day im, days 0 to 9	5/20	12/14
6-MP, 50 mg/kg/day ip, days 0 to 9	9/12	11/12
6-MP, 37.5 mg/kg/day im, days 0 to 9	5/12	9/10
6-MP, 25 mg/kg/day im, days 0 to 9	8/11	7/9
BCG only	24/30	24/26
Normal animals not infected with BCG	0/13	0/7

* Reactions read 24 hours after intradermal injection of 0.005 mg PPD in 0.1 ml saline. Only reactions with a mean diameter of induration of 10 mm or greater are recorded as positive.

RESULTS

Effect of 6-MP Treatment on Cutaneous Hypersensitivity.—Table I shows the effect of 6-MP treatment in major experimental groups. It can be seen that 6-MP in the dosage of 50 mg/kg/day, im, begun on the day of BCG stimulation suppressed the development of delayed hypersensitivity during the period of drug administration. When treatment was discontinued, however, tuberculin hypersensitivity developed within 10 days. Lower doses of 6-MP given intramuscularly were less effective in suppressing delayed hypersensitivity and intraperitoneal injections had no suppressive effect.

The range of skin reactions in the 6-MP–treated and control guinea pigs is given in Table II. Fourteen guinea pigs treated with 6-MP are compared with 10 control animals. While more control guinea pigs show delayed hypersensitivity than the 6-MP–treated animals on day 10, the two groups are indistinguishable

TABLE II

Effect of 6-MP on the Development of Delayed Hypersensitivity in Guinea Pigs Infected with BCG

Diameter of induration of skin reactions at 24 hours after skin test with 0.005 mg PPD			
6-MP, 50 mg/kg/day im, days 0 to 9		Untreated	
Test on day 10*	Test on day 19	Test on day 10	Test on day 19
mm	*mm*	*mm*	*mm*
15 × 15	15 × 15	17 × 25	12 × 30
10 × 12	12 × 12	14 × 20	12 × 20
10 × 10	10 × 10	14 × 15	15 × 18
9 × 12	14 × 14	12 × 12	20 × 20
8 × 10	15 × 20	12 × 12	15 × 15
—	20 × 20	10 × 12	15 × 15
—	15 × 20	10 × 10	12 × 15
—	15 × 15	9 × 12	9 × 15
—	12 × 15	—	14 × 14
—	12 × 15	—	—
—	10 × 10		
—	9 × 25		
—	7 × 8		
—	—		
Positive reactions‡.....4/14	12/14	8/10	9/10

* Reactions of less than 7 × 7 mm are given as negative (−), for normal animals regularly showed reactions up to this size when tested with PPD.

‡ Only reactions with a mean diameter of induration of 10 mm or greater are recorded as positive.

TABLE III

Skin Reactions of Guinea Pigs Tested with PPD for Cutaneous Hypersensitivity

Treatment	Fraction of tested animals having positive reactions
	Day 10 after BCG infection*
Fasted days 0 to 11 after BCG infection.....	11/15‡
BCG only.....	9/12
Normal animals.....	0/6

* Reactions read 24 hours after intradermal injection of 0.005 mg PPD in 0.1 ml saline. Only reactions with a mean diameter of induration of 10 mm or greater are recorded as positive.

‡ Six guinea pigs died before they could be skin-tested on the 10th day after fasting was started.

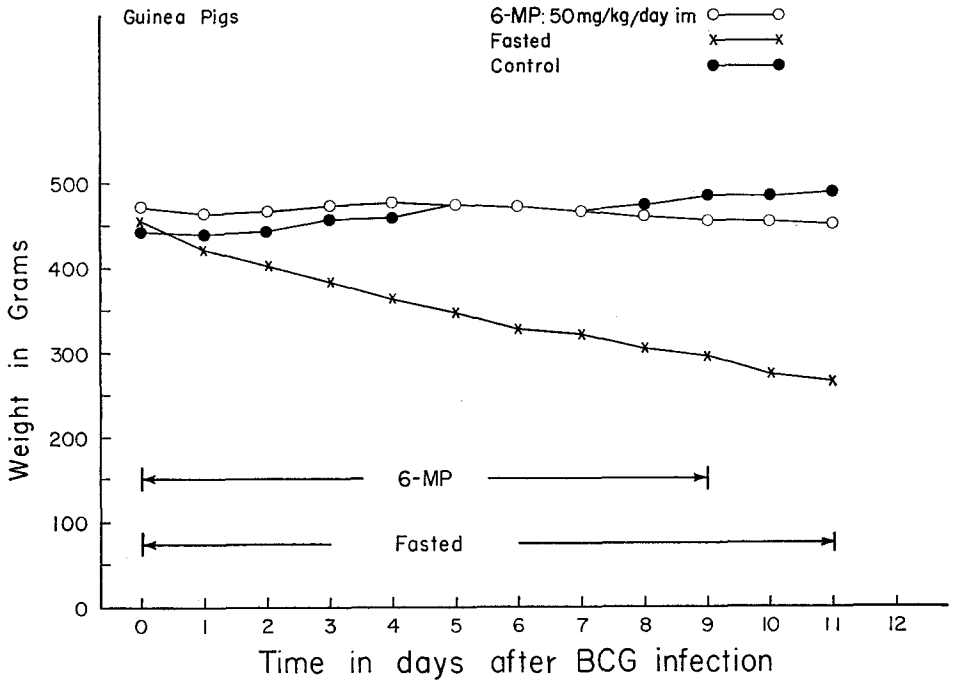

FIG. 1. The effect of fasting and 6-MP administration on weight loss in BCG-infected guinea pigs.

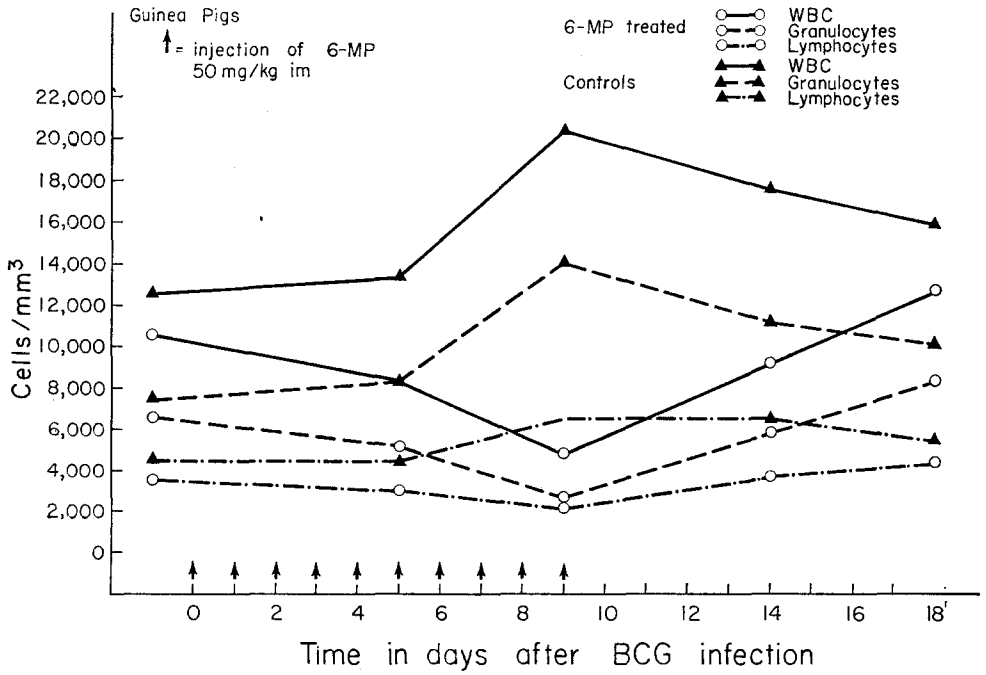

FIG. 2. The effect of 6-MP on circulating leukocytes in BCG-infected guinea pigs.

in the number and size of reactions 10 days after treatment was discontinued (day 19).

Weight loss accompanies administration of 6-MP and might be considered a sign that 6-MP acts as a non-specific toxic agent through debilitation and fasting. Table III shows that fasted guinea pigs develop delayed hypersensitivity as readily as the control animals. In these fasted animals the net weight loss was much greater than in animals receiving the highest dose of 6-MP (Fig. 1) but delayed hypersensitivity was not affected.

Effect of 6-MP on Circulating Leukocytes.—A slight depression of circulating leukocytes was noted in BCG-stimulated guinea pigs given 50 mg/kg/day of 6-MP and the major effect was on the number of neutrophils. For contrast, the hematologic data for a group of guinea pigs receiving only BCG stimulation are also given in Fig. 2.

Toxicity of 6-MP.—Of 20 guinea pigs stimulated with BCG and then treated for 9 days with 6-MP (50 mg/kg/day, im), 6 died between days 10 and 19. During this same period 2 of 12 animals receiving 37.5 mg/kg/day, im, and 2 of 11 receiving 25 mg/kg/day, im, died. Only 2 of 30 control animals sensitized with BCG died in the same period and 2 others were sacrificed for histologic studies. Greater toxicity was observed in guinea pigs given 10 daily injections of 6-MP (50 mg/kg/day, im) but not stimulated with BCG. Five of eight guinea pigs so treated died by the 16th day after the first injection.

DISCUSSION

Tuberculin hypersensitivity following infection with BCG in guinea pigs is classical delayed hypersensitivity: circulating antibody cannot be detected and the hypersensitivity is readily transferred to normal guinea pigs with leukocytes from sensitized donors (5). Cellular infiltration at skin test sites is predominantly mononuclear as contrasted with the polymorphonuclear invasion present in Arthus hypersensitivity (6).

In studying the mechanism by which delayed hypersensitivity develops, a wide range of agents which effectively suppress the sensitization process have been investigated. These include x-irradiation (7), hormones (8), folic acid antagonists (1, 9), large doses of antigen (10), and antilymphocyte serum (11). The experiments reported here indicate that another type of chemical, a nucleic acid antimetabolite, also suppresses the development of delayed hypersensitivity during the period of drug administration.

Schwartz *et al.* found that antibody production in the rabbit was inversely proportional to 6-MP dosage (2). Subsequently, it has been shown that 6-MP prevents the secondary immune reaction (12), prolongs homograft survival (13), and prevents the development of experimental allergic encephalomyelitis (14) during the period of drug administration.

Although large doses of 6-MP were required to inhibit the development of

tuberculin hypersensitivity, most of the BCG-sensitized guinea pigs tolerated the drug quite well. There was minimal weight loss, moderate mortality, and relatively mild leukopenia. Moreover, the same animals developed delayed hypersensitivity reactions when administration of 6-MP was discontinued. The possibility that 6-MP had allowed dissemination of the mycobacteria was considered, for the absence of delayed sensitivity might have followed overwhelming infection. Histologic studies of the lungs and livers of guinea pigs sacrificed after BCG infection failed to show any consistent difference between control and 6-MP–treated animals, however.

The present study differs in several ways from previously reported investigations of the effect of 6-MP on delayed hypersensitivity (3, 4). Our results indicate that intramuscular injection of 6-MP is much more effective in suppressing delayed hypersensitivity that intraperitoneal injection and that at least 37.5 mg/kg/day must be administered to the guinea pigs. We also used a different method of antigenic stimulation and this may account for the different findings. Salvin studied the effect of 6-MP in guinea pigs given a protein antigen in an adjuvant emulsion (3). In contrast, we sensitized guinea pigs with living BCG in a saline suspension and in this way avoided the intensive stimulation which follows from adjuvant administration. In antibody formation also, the effect of 6-MP is much greater in animals sensitized without adjuvants (15).

Genghof and Battisto concluded from their experiments that 6-MP affected neither delayed hypersensitivity nor antibody formation in the guinea pig and ascribed this to a species difference in response to the drug (4). In view of the findings presented here, a more likely explanation is that they used dosages (9 mg/kg/day, im, and 40 mg/kg/day, ip) which were inadequate to obtain an anti-immunologic effect in the guinea pig. When adequate doses are administered, 6-MP suppresses both antibody formation (16) and delayed hypersensitivity in the guinea pig.

SUMMARY

The development of tuberculin hypersensitivity in guinea pigs after BCG stimulation was suppressed by intramuscular administration of 50 mg/kg/day of 6-mercaptopurine started at the time of stimulation. Fasting of guinea pigs after BCG stimulation had no effect on the development of tuberculin hypersensitivity.

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