The Morphologic Responses of the Lymphoid System to Homografts. II. The Effects of Antimetabolites

By JANINE A. ANDRÉ,* ROBERT S. SCHWARTZ, W. J. MITUS AND WILLIAM DAMESHEK

IN A PREVIOUS paper, the morphology of the lymphoid system of the rabbit during the development of transplantation immunity was described.[1] The outstanding feature of the reaction was the proliferation of primitive cells (hemocytoblasts), which, although they first appeared in the lymph node contiguous to the graft, were eventually seen throughout the entire lymphatic apparatus of the animal. The growth of hemocytoblasts was associated with enlargement of the germinal centers and lymphoid follicles of the lymph nodes and spleen, and with effacement of normal landmarks. Although these lymphoid changes were most prominent at the peak of the reaction within the graft, they persisted for many days, especially after the rejection of a second set graft. The results of this study were considered to be consistent with the concept that immunity to a local graft eventually becomes a generalized reaction. They also suggested that a second set phenomenon may represent an anamnestic response, rather than a state of sustained immunity, which is "revealed" by the second graft.

The present report describes the effects of the antimetabolites 6-mercaptopurine (6-MP) and thioguanine on these responses. It was found that these agents abolished the cellular proliferation described above; for as long as the growth of hemocytoblasts was prevented by the drugs, the homograft reaction did not occur. On the other hand, permanent suppression of the homograft reaction did not occur. Graft rejection despite the continued administration of 6-MP was marked by an explosive proliferation of hemocytoblasts and a striking hyperplasia of the germinal centers and follicles of the lymph nodes and spleen. The hemocytoblasts which appeared in these rabbits were considered to be "drug resistant" and analogous to resistant bacterial or leukemic cell populations which thrive despite the continued presence of antimetabolites.

MATERIALS AND METHODS

The selection and care of the rabbits, the technic of skin grafting and the cytologic and histologic methods were the same as those previously described.[1] White blood counts and microhematocrits, performed at weekly intervals, were done by standard methods. Solutions of 6-MP and thioguanine were prepared daily by dissolving 100 mg. of the powdered antimetabolite° in 1 ml. of 1 N NaOH; the desired concentration for injection (usually

From the Blood Research Laboratory, Pratt Clinic-New England Center Hospital, and the Department of Medicine, Tufts University School of Medicine, Boston, Mass.

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Table 1

Group	Number of Animals	Type of Homograft	Drug	Dose of Drug*	Timing of Drug Administration	Days Sacrificed
A	25	none	TG	1.0	———	2,4,6,8,10
B	25	none	TG	0.5	———	3,5,10,12,15
C	25	1st set	TG	1.0	beginning on day of graft application	2,4,6,8,10
D	25	1st set	TG	0.5	beginning on day of graft application	3,5,10,12,15
E	25	1st set	6-MP	12.0	beginning on day of graft application	5,10,15,20,25
F	6	1st set	6-MP	12.0	starting 8 days before graft application	8
G	6	1st set	6-MP	12.0	starting 2 days after application of graft	8
H	6	1st set	6-MP	12.0	starting 3 days after application of graft	8
I	6	1st set	6-MP	12.0	starting 4 days after application of graft	8
J	45	2nd set	6-MP	12.0	beginning on day of 2nd graft application	1,2,3,4,5,10, 15,20,25
K	6	2nd set	6-MP	12.0	starting 8 days before application of 2nd graft	8
L	9	1st set	6-MP	12.0	beginning on day of graft application	at earliest sign of graft rejection
M	13	1st set	6-MP	12.0	beginning on day of graft application	at height of graft rejection
N	8	1st set	6-MP	12.0	beginning on day of graft application; ending with graft rejection	10 days after graft rejection
O	11	1st set	6 MP	12.0	beginning on day of graft application until day of sacrifice	10 days after graft rejection
P	8	2nd set	6-MP	12.0	beginning on day of 1st graft application; ending with rejection of 1st set	on rejection of 2nd set graft
Q	6	repeated homograft	6-MP	12.0	beginning on day of 1st graft application to day of sacrifice	20
R	6	repeated homograft	6-MP	6.0	beginning on day of 1st graft application to day of sacrifice	26

*mg./Kg./day.

12 mg./ml. for 6-MP and 1 mg./ml. for thioguanine) was made by appropriate dilution of the concentrated solution with physiologic saline. All injections of drugs were made subcutaneously. Two hundred and sixty-one rabbits divided into eighteen groups were studied. These are shown in table 1. Because thioguanine proved to be extremely toxic in rabbits, extensive studies were not made with this agent.

Results

Treatment of non-grafted rabbits with thioguanine in a dose of 1.0 mg./Kg./day (Group A) caused moderate atrophy of lymph nodes within six to eight days. The gross appearance of the nodes was frequently hemorrhagic and hyperemia was seen in the microscopic sections. Imprints of these nodes showed a reduction in the percentage of hemocytoblasts to 0.5 per cent (normal: 1 per cent). No changes were seen in the spleen. Group B, which was

treated with 0.5 mg./Kg./day, developed similar changes after 10 to 12 days of drug administration; hemorrhage was occasionally seen in the lymph nodes and spleen of this group after 15 days of treatment.

Groups C, D and E, to be described presently, consisted of animals which had retained the homograft during the course of the experiment. Therefore, the gross appearance of the homograft was indicative of a "take" in all instances. Indications of a successful take included absence of edema, cyanosis, or necrosis, proper healing of the surgical wound, and ultimate growth of hair. The microscopic sections of these homografts resembled normal skin, with intact epithelium, normal dermal appendages and morphologic signs of blood flow. In contrast to what was observed during the rejection of first set homografts in normal animals, rabbits treated with thioguanine in doses of 1.0 mg./Kg./day or with 6-MP in a dose of 12 mg./Kg./day (Groups C, D, and E) developed only minimal enlargement of the germinal centers and lymph follicles on the fifth to seventh days. At no time was the lymphatic architecture disrupted as was observed in the control group. As the treatment was continued, atrophy of the lymph nodes occurred and the germinal centers and lymphoid follicles appeared shrunken (fig. 1). Prolonged treatment with these drugs produced striking hyperemia of the lymph nodes, particularly in those draining the graft and in the spleen. The number of hemocytoblasts never exceeded 1.5 per cent during the course of these experiments (fig. 2). Lymphoblasts and medium sized lymphocytes were greatly depleted, particularly in the lymph node draining the homograft. Moderate weight loss, anemia and leukopenia occurred in all animals retaining their homografts.

Treatment of the animals for seven days with 6-MP prior to the application of the homograft (Group F) did not alter the capacity of this group of rabbits to react to and to reject the homograft. Rejection of the graft occurred at the same time as in control animals and the intensity of the reaction in the lymph nodes and spleens was the same as in untreated rabbits. When treatment with 6-MP was delayed for two (Group G), three (Group H) or four (Group I) days after application of the graft, it was ineffective in preventing graft destruction. The cellular proliferation in the lymph nodes of these rabbits was unimpaired and the grafts showed typical gross and microscopic signs of rejection.

The second set homograft response was unaffected by 6-MP (Group J). No prolongation of the rejection time was observed, and the signs of rejection in the graft were undiminished by the antimetabolite treatment. The histologic sections of the lymph nodes and spleen were comparable to those seen in untreated rabbits rejecting second set homografts. However, the percentage of hemocytoblasts, particularly in the draining lymph node, was reduced to 4.8 per cent, even at the height of the homograft reaction (fig. 3). Proplasmacytes were increased (4.6 per cent) in the draining lymph node. Pretreatment of the second set response (Group K) neither prolonged the life of the homograft nor prevented the lymphoid responses to it.

No *permanent* homograft "takes" were observed in these experiments. Rabbits rejecting their grafts despite continuous 6-MP treatment were divided into the following groups: nine animals (Group L) which were sacrificed at the

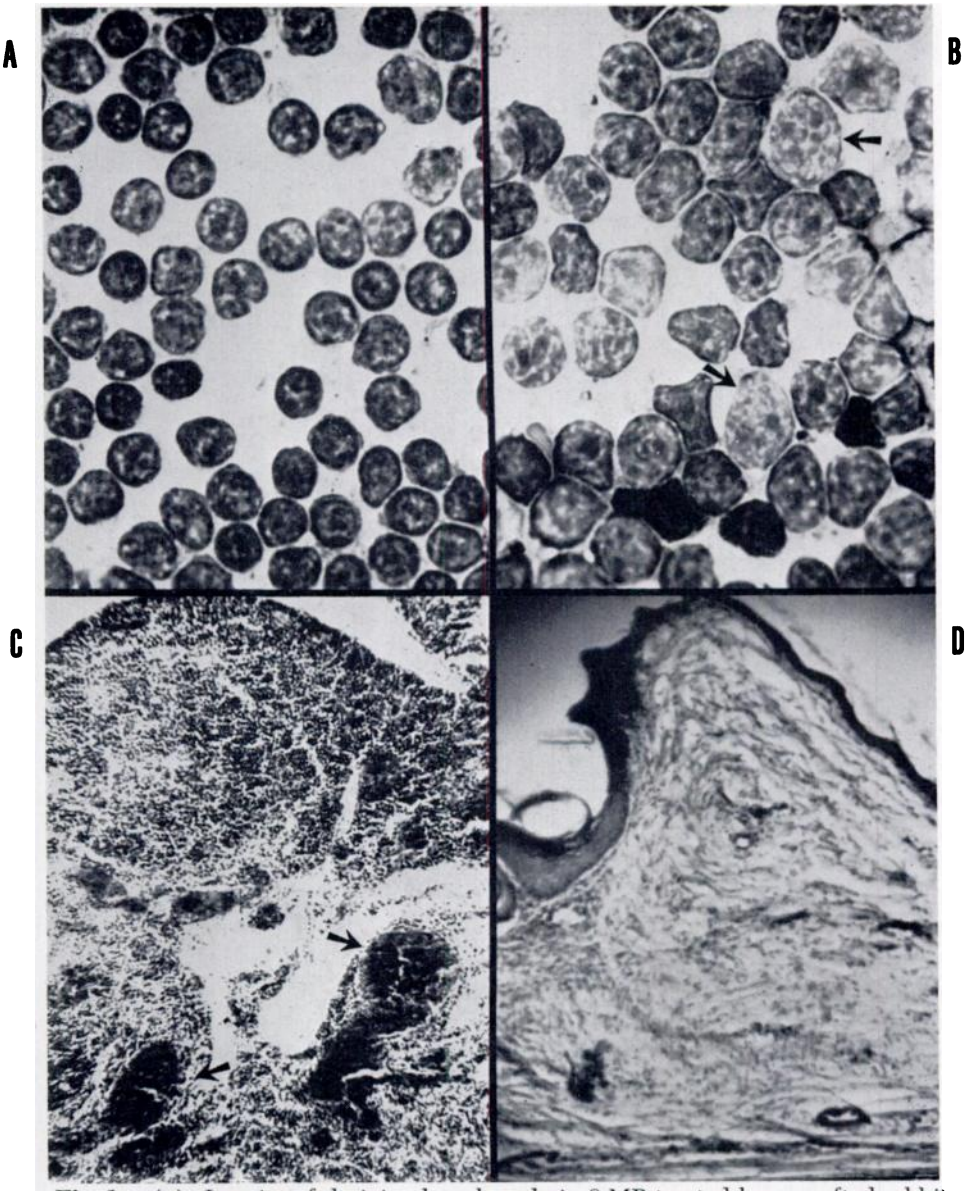

Fig. 1.—(a) Imprint of draining lymph node in 6-MP treated homografted rabbit. There are no hemocytoblasts and the cellular constituents are almost exclusively small lymphocytes. (b) Imprint of contralateral lymph node in 6-MP treated homografted animal. Hemocytoblasts are absent but medium size lymphocytes are present (arrows). (c) Section from lymph node of figure 1a showing atrophy and hyperemia. (d) Nine-day-old skin graft from 6-MP treated rabbit. There is no evidence of rejection.

first macroscopic sign of graft rejection (usually cyanosis and edema); and 13 animals (Group M) which were sacrificed at the height of gross evidence of graft rejection. A characteristic feature of the reaction within the grafts in these two groups was the presence of widespread hemorrhage, which was

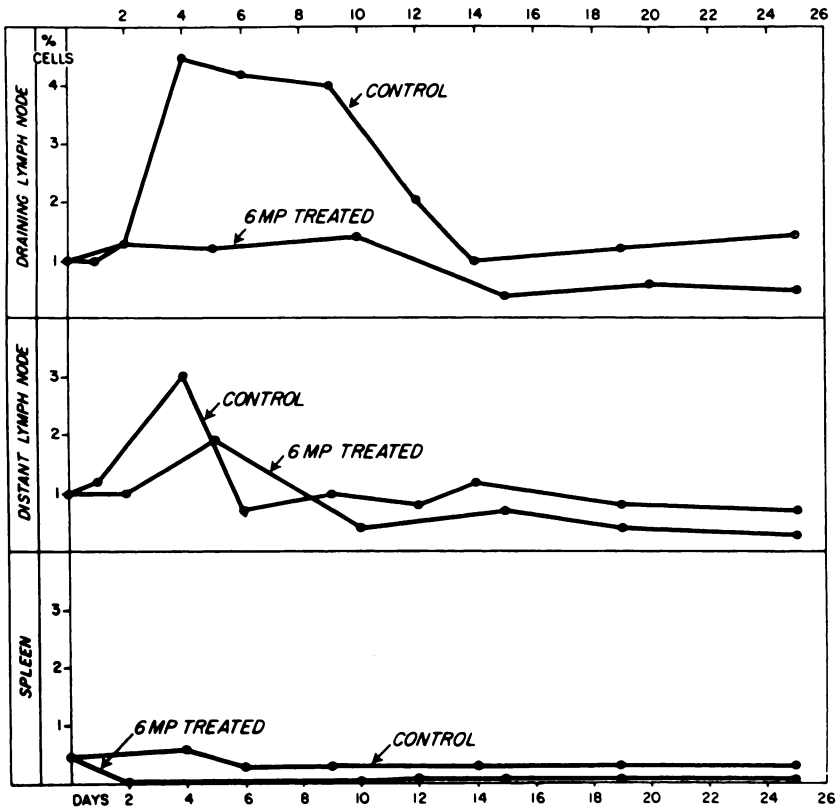

Fig. 2.—Effect of 6-mercaptopurine on hemocytoblast responses to first set skin homografts.

never seen in untreated animals. In sharp contrast to untreated animals, in which the cellular reaction in the graft was almost exclusively lymphocytic, clusters of primitive cells, presumably hemocytoblasts, were seen within the dermal layers of their grafts. In both groups, and particularly in Group M, the draining lymph node was markedly enlarged and stony hard. In the living animal, it was easily palpated at the base of the ear.

Animals studied at the first sign of graft rejection (Group L) showed striking hyperplasia of the germinal centers and lymphoid follicles in the draining node, as well as hyperemia and hemorrhage. The contralateral node showed similar changes, but to a lesser degree. The spleen showed moderate follicular hyperplasia.

In animals of Group M, which were studied at the height of the homograft reaction, the lymph node contiguous to the homograft showed the most striking hyperplasia encountered in these experiments (fig. 4f). The germinal centers were enormously enlarged and occupied almost the entire follicle, which was itself greatly distended. Architectural landmarks were partially effaced and hyperemia and hemorrhage were present. The contralateral node showed similar changes, but to a lesser degree. Large germinal centers and follicles were also seen in the spleen; these changes were never seen in the

Table 2.—*Cellular Constituents of the Lymphoid Tissues in Rabbits Treated with 6-Mercaptopurine During a First Set Homograft Reaction*

	Hemocytoblasts			Proplasma Cells			Plasma Cells			Eosinophils			Reticulum Cells		
Day	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen
0	1.0 ± 0.7	1.0 ± 0.7	0.5 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.4	0.5 ± 0.6	0.5 ± 0.6	0.3 ± 0.7	0.7 ± 0.8	0.7 ± 0.8	3.0 ± 0.8	4.7 ± 1.1	4.7 ± 1.1	6.1 ± 1.1
2	1.3 ± 1.8	1.0 ± 0.9	0.0 ± 0.0	0.1 ± 1.1	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.6	0.2 ± 0.6	0.1 ± 0.1	1.4 ± 0.5	0.8 ± 1.2	17.2 ± 6.5	3.1 ± 0.6	3.0 ± 0.6	5.4 ± 0.6
5	1.2 ± 1.0	1.9 ± 1.4	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.5 ± 0.7	0.5 ± 0.9	0.2 ± 1.0	1.7 ± 1.2	1.3 ± 1.8	9.8 ± 1.8	3.4 ± 0.5	2.8 ± 0.9	5.4 ± 0.5
10	1.4 ± 0.7	0.4 ± 0.6	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 1.0	0.2 ± 0.7	0.1 ± 0.8	0.3 ± 0.5	0.3 ± 0.7	6.2 ± 1.0	3.1 ± 0.1	3.2 ± 0.5	5.8 ± 0.5
15	0.4 ± 0.6	0.7 ± 0.6	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 1.4	0.1 ± 0.4	0.5 ± 0.7	0.5 ± 0.5	3.7 ± 2.8	2.9 ± 0.7	3.5 ± 0.6	5.6 ± 0.8
20	0.6 ± 0.5	0.4 ± 0.6	0.0 ± 0.0	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 1.0	1.2 ± 0.8	0.8 ± 1.3	2.5 ± 0.5	2.8 ± 0.5	5.6 ± 0.5
25	0.5 ± 0.5	0.3 ± 0.5	0.0 ± 0.0	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.5	1.2 ± 1.9	0.6 ± 0.5	0.4 ± 0.5	2.0 ± 1.4	2.7 ± 0.6	3.3 ± 0.4	5.7 ± 0.1

Table 3.—*Cellular Constituents of the Lymphoid Tissues in Rabbits Treated with 6-Mercaptopurine During a Second Set Homograft Reaction*

	Hemocytoblasts			Proplasma Cells			Plasma Cells			Eosinophils			Reticulum Cells		
Day	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen
0	1.0 ± 0.7	1.0 ± 0.7	0.5 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.4	0.5 ± 0.6	0.5 ± 0.6	0.3 ± 0.7	0.7 ± 0.8	0.7 ± 0.8	3.0 ± 0.8	4.7 ± 1.1	4.7 ± 1.1	6.1 ± 1.1
1	2.4 ± 1.9	1.4 ± 0.9	0.6 ± 0.6	0.6 ± 0.7	1.0 ± 1.0	0.6 ± 0.6	1.8 ± 1.7	1.2 ± 0.9	2.6 ± 1.6	1.3 ± 1.9	0.5 ± 0.6	5.0 ± 2.1	3.2 ± 0.4	3.1 ± 0.1	5.0 ± 0.7
2	2.2 ± 0.7	1.6 ± 1.2	0.7 ± 0.6	1.5 ± 1.2	1.5 ± 1.2	0.7 ± 0.6	1.3 ± 1.0	1.8 ± 1.7	1.8 ± 1.6	1.1 ± 1.7	1.3 ± 1.3	10.2 ± 4.7	3.2 ± 0.4	3.4 ± 0.5	5.0 ± 0.7
4	2.2 ± 1.0	0.7 ± 0.8	0.8 ± 0.7	2.4 ± 2.4	0.9 ± 1.3	1.0 ± 1.2	1.6 ± 1.3	1.4 ± 1.2	0.5 ± 0.7	1.0 ± 1.0	2.0 ± 1.8	6.2 ± 3.9	3.3 ± 0.4	3.3 ± 0.5	5.5 ± 0.5
7	4.8 ± 1.3	1.0 ± 0.8	0.8 ± 0.7	4.6 ± 0.9	0.7 ± 0.6	0.8 ± 0.6	0.6 ± 0.6	0.9 ± 0.8	1.4 ± 0.9	1.9 ± 1.8	3.4 ± 0.9	5.2 ± 0.8	3.4 ± 1.5	3.4 ± 0.9	5.2 ± 0.8
10	0.6 ± 0.5	0.9 ± 0.8	0.3 ± 0.4	0.6 ± 0.8	0.9 ± 0.7	0.4 ± 0.7	0.6 ± 0.6	0.8 ± 1.2	0.9 ± 0.6	0.9 ± 0.9	0.5 ± 0.7	5.0 ± 0.8	3.3 ± 0.5	3.3 ± 0.8	5.5 ± 0.6
15	0.5 ± 0.5	0.9 ± 0.6	0.4 ± 0.5	0.2 ± 0.5	0.4 ± 0.6	0.3 ± 0.6	0.6 ± 0.8	0.4 ± 0.6	0.8 ± 0.7	2.5 ± 0.8	1.8 ± 1.6	6.4 ± 1.6	3.1 ± 0.5	3.1 ± 0.8	5.8 ± 0.6
20	0.7 ± 0.3	0.9 ± 0.5	0.3 ± 0.4	0.2 ± 0.4	0.4 ± 0.6	0.2 ± 0.5	0.7 ± 0.5	0.4 ± 0.6	0.7 ± 0.7	2.0 ± 1.3	1.6 ± 1.0	5.9 ± 0.9	2.0 ± 1.3	3.2 ± 0.7	6.1 ± 0.6
25	0.5 ± 0.5	0.9 ± 0.6	0.3 ± 0.4	0.4 ± 0.4	0.3 ± 0.6	0.3 ± 0.7	0.6 ± 0.4	0.4 ± 0.6	0.7 ± 0.7	2.2 ± 0.8	1.5 ± 1.2	6.1 ± 1.2	2.8 ± 0.7	3.3 ± 0.7	5.9 ± 0.8

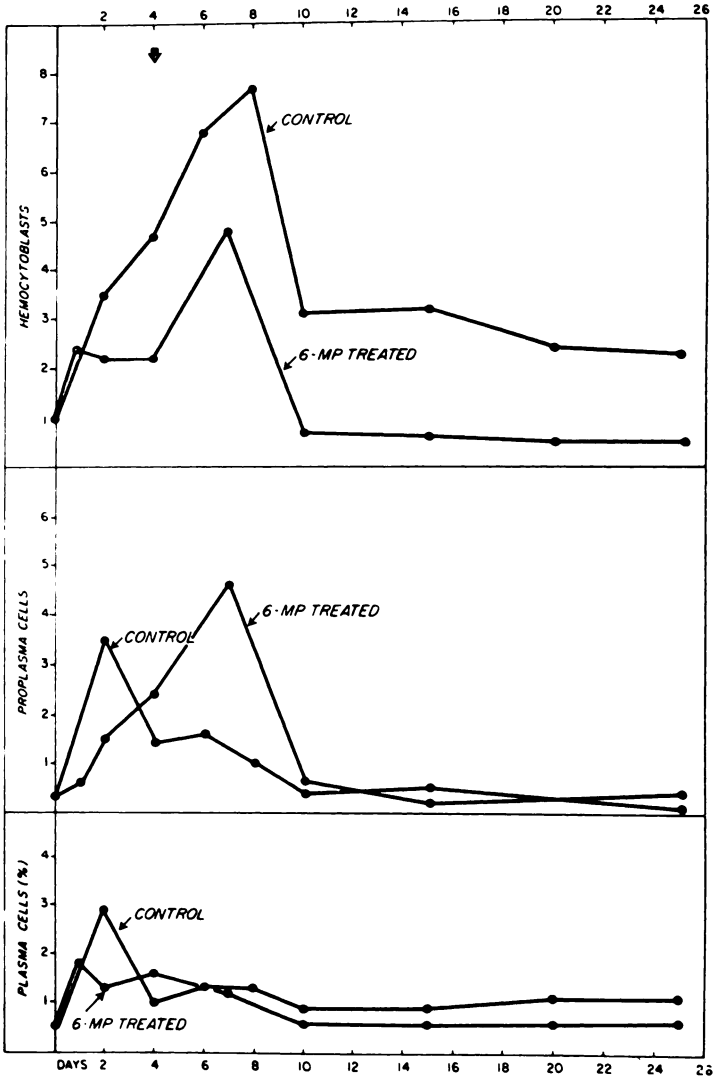

Fig. 3.—Comparison of cellular responses in lymph nodes draining second set homografts in control and 6-MP treated rabbits.

spleen of untreated animals in this stage of the first set homograft reaction. Imprints of the lymph nodes and spleen showed numerous hemocytoblasts; in one animal, they constituted 12.6 per cent of the cell population (fig. 5). The hemocytoblasts which appeared under these circumstances were larger, and perhaps more primitive than those usually seen (fig. 4a, b, c).

In an attempt to elucidate the factors responsible for the extreme morphologic changes in animals rejecting first set homografts while under treatment with 6-MP, four additional groups of rabbits were examined. In eight animals, the 6-MP was discontinued when rejection of the homograft was clearly evident, and 10 days later the animals were killed (Group N). Their lymph nodes

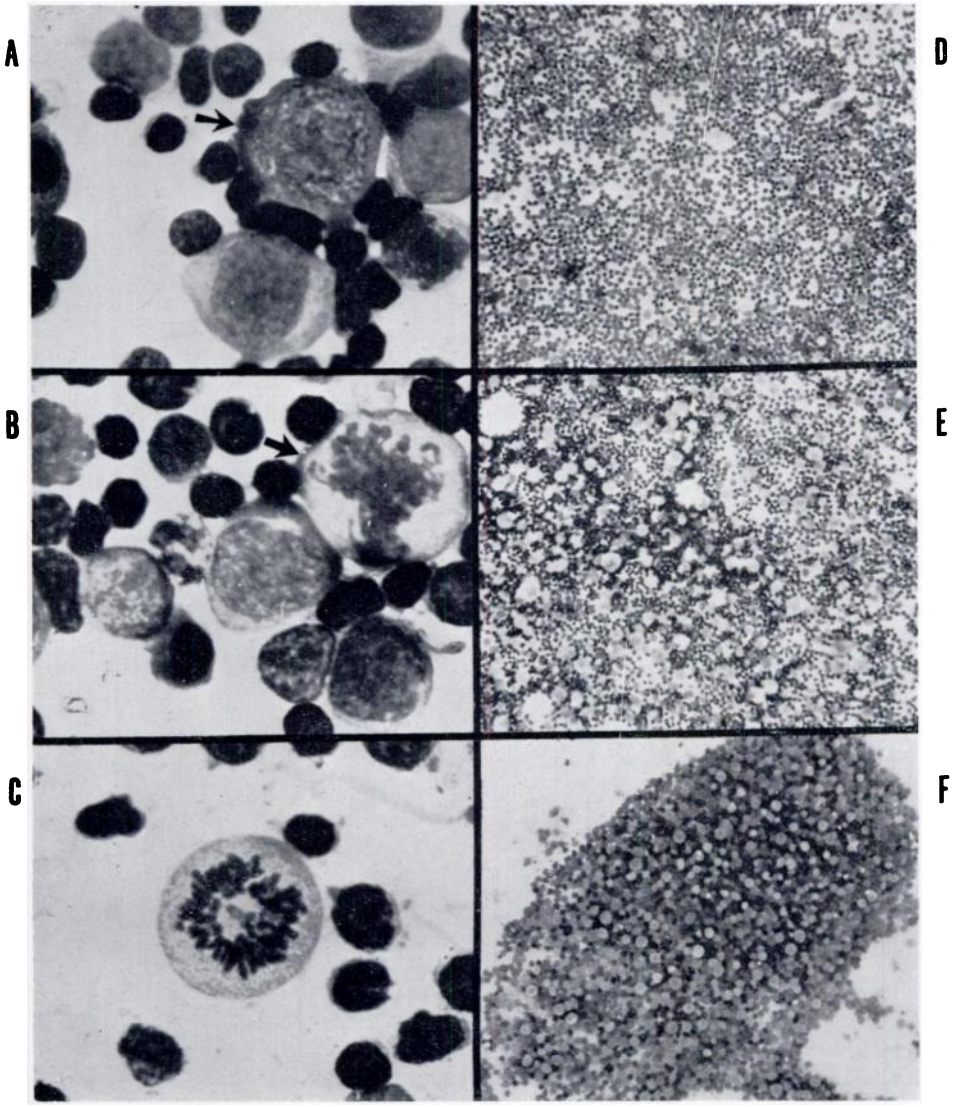

Fig. 4.—(a, b, c) "6-MP resistant hemocytoblasts". Note the coarse nuclear chromatin of the cell in 4a (arrow) and the mitotic figures (4b, 4c). **(d)** Draining lymph node in an untreated rabbit at the peak of the first set reaction. **(e)** Draining lymph node of an untreated rabbit at the peak of the second set reaction. The increase in hemocytoblasts is evident even at this low power. **(f)** Draining lymph node in a "6-MP resistant animal" at the peak of the first set reaction. Note the enormous increase in hemocytoblasts.

and spleens showed persistent hyperplasia of the germinal centers and follicles, but to a lesser extent than in the previous group. The imprint preparations of this group showed a considerable diminution of hemocytoblasts (to 2 per cent), but the previously noted primitivity persisted. Eleven rabbits were given 6-MP continuously and were killed 10 days after rejection of the homo-

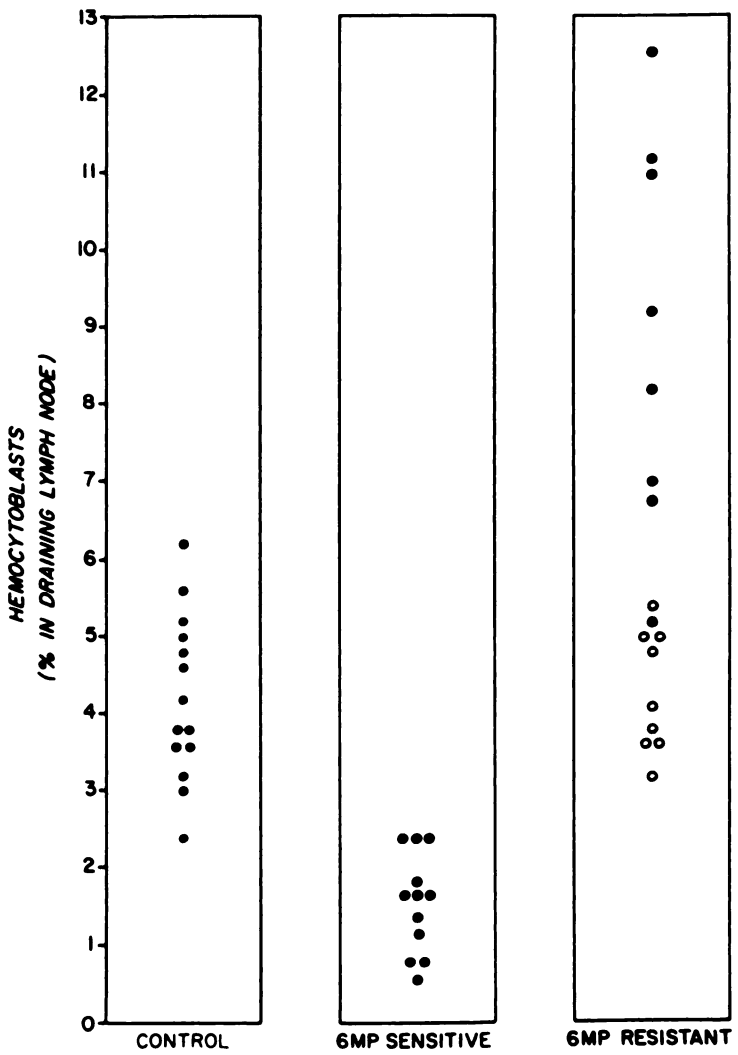

Fig. 5.—Cellular responses to first set homografts in 6-MP-sensitive and 6-MP-resistant rabbits.

graft (Group O). Their tissues also showed lymphoid hypertrophy—more marked than in Group N where 6-MP treatment was stopped when the graft was rejected, but less than in Group M. In eight animals, the 6-MP was stopped at the time of homograft rejection and, three weeks later, a second graft was applied, but without further 6-MP treatment (Group P). At the peak of the rejection of the second graft, the lymph nodes showed marked hyperplasia, hyperemia and hemorrhages, while the splenic follicles of this group were only slightly enlarged. These changes were judged to be somewhat more pronounced than those of the untreated rabbits rejecting a second set homograft. In 12 animals, multiple (three to five) homografts from a single donor were applied and 6-MP was administered continuously. Six of them received 12

mg./Kg./day (Group Q) and six were given 6 mg./Kg./day (Group R). Both Groups Q and R developed marked enlargement of the germinal centers, especially in the node draining the graft. Hyperemia was slight and hemorrhage was absent. Follicular hyperplasia and triple zoning were striking in the spleen. These changes were greater than those which occurred in a group of untreated rabbits which had been grafted with three to five homografts from a single donor.

Discussion

It has been previously demonstrated that 6-MP suppresses, at least temporarily, transplantation immunity. Thus it was shown in one study that the mean rejection time of skin homografts in rabbits could be prolonged to 17.8 days[2] and to 24.3 days in another.[3] The drug was given in a dose of 12 mg./Kg./day in both experiments, subcutaneously in the former and intravenously in the latter. The rejection of canine renal homografts[4,5] has also been delayed by the administration of this agent. Other forms of tissue immunity have been suppressed, including secondary bone marrow disease[6] and tumor immunity in mice[7] by amethopterin, and skin grafts in chickens by 6-MP.[8] The latter two studies demonstrated the induction by these drugs of a state closely resembling acquired immunologic tolerance, a phenomenon previously reported with purified protein antigens.[9]

In some of the animals of the previously reported series, 6-MP had no apparent effect, the grafts being rejected at the usual time. Furthermore, several investigators noticed that although a homograft may "take" as the result of 6-MP treatment, it eventually broke down in spite of continued drug administration. Histologic examination of these rejected grafts was entirely compatible with a homograft reaction.

The purposes of the present experiments were to investigate the following problems: (1) What is the appearance of the lymphoid system of animals retaining homografts because of 6-MP treatment? (2) What morphologic changes occur in the lymph nodes and spleens of animals rejecting homografts in the face of continuous 6-MP treatment?

The lymphoid atrophy seen in non-grafted rabbits treated with thioguanine was generalized and extensive. On the other hand, the effects of this agent on the lymphatic tissue of rats and dogs was reported as minimal although signs of myelotoxicity occurred;[10] however, Zukoski, Lee and Hume found that 6-MP caused lymph follicle destruction in dogs bearing renal homografts.[5] This species selectivity may explain the failure of 6-MP to inhibit the immune responses in rats[11] and guinea pigs.[12] The marked toxicity of thioguanine as compared to 6-MP in the rabbit is a further example of species differences, since these agents are of approximately equal toxicity in man and dogs. Toxic side effects do not always parallel the ability of an agent to inhibit immune mechanisms, but the immune inhibitory effect of thioguanine was most pronounced when it was given at toxic doses. The differences in toxicity of these agents in various species may reflect differences in their metabolic degradation; for example, the chicken can tolerate up to 400 mg./

Kg./day of 6-MP, while man often develops toxicity at a dosage of 2.5 mg./Kg./day.

The characteristic lymphatic response to a skin homograft in the rabbit is enlargement of the germinal centers and lymph follicles together with the proliferation of large, primitive appearing cells called hemocytoblasts. The reaction within the lymph nodes parallels the development of transplantation immunity and reaches its peak coincident with the full expression of immunity within the homograft. When 6-MP was effective in preventing homograft rejection, none of these changes occurred. In contrast to the lymphoid hyperplasia seen in the untreated animal, the outstanding feature was extensive lymphoid atrophy especially marked in the lymph node draining the graft; there, the germinal centers and follicles were reduced in size, hyperemia and hemorrhage were prominent, and the cell population was composed almost exclusively of small lymphocytes. Distant lymph nodes and the spleen, although showing a considerable atrophy, contained many more lymphoblasts and meduim size lymphocytes. The differential effects of 6-MP on the draining and distant lymph nodes may be due to differences in metabolic activity; such differences were reported by Pierce, Meeker and Varco who found considerable increases in nucleic acid synthesis of lymph nodes draining homografts, but not in the spleen.[13]

Our previous studies emphasized the conspicuous role of the hemocytoblast in transplantation immunity. This cell, thought to be a precursor of both lymphocytes and plasmocytes, arose within widespread lymphatic centers in response to a locally applied homograft, proliferated at maximum intensity at the time of maximum homograft distress and subsided as the graft was replaced by scar tissue. Although likened to "effector organelles," the mechanism whereby their effect (homograft rejection) was produced, was not elucidated. Since hemocytoblasts were never found within the graft, the possible participation of a third element (daughter lymphocyte, humoral agent), could be surmised. The essential part played by these cells in transplantation immunity was also delineated in the present experiments; homograft rejection did not occur in the absence of hemocytoblasts. It appears then that 6-MP retards graft rejection by inhibiting the growth of hemocytoblasts. As has been previously shown, the timing of antigen administration and drug therapy is crucial. Sterzl has concluded that 6-MP is effective only if given during the induction period of antibody formation.[14] The results in Groups F, G, H, K, and K support these studies, since they showed that pretreatment or delayed treatment with 6-MP neither delayed homograft rejection nor suppressed the morphologic consequences of homograft application. Once initiated, the proliferation of hemocytoblasts could not be stopped by 6-MP. It would thus appear that once the metabolic "machinery" for antibody formation is set in motion, 6-MP is without effect. However, Meeker recently showed that treatment of rabbits with large doses of antigen and large doses of 6-MP could inhibit an anamnestic response.[15]

Cortisone, which has lymphocytolytic properties, and which suppresses immune responses, has also been shown to prolong the life of skin homografts in

rabbits. Billingham, Krohn and Medawar[16,17] demonstrated that not only did systemically administered cortisone suppress transplantation immunity, but that small amounts of cortisone applied directly to the skin graft also prolonged its life. The experiments of Scothorne[18-20] substantiated these findings and further demonstrated that treatment with cortisone, systemically or locally, was accompanied by a marked reduction in "large lymphoid cells" in the draining lymph node. On the basis of this work he suggested that the large lymphoid cell (probably the same cell as the "hemocytoblast" described here) was an active participant in transplantation immunity.

The morphologic changes found in the lymph nodes and spleens of animals rejecting homografts while on 6-MP treatment were unexpected. The degree of lymphoid hyperplasia in those animals, which were rejecting *first* set homografts, exceeded the responses observed during the peak of the *second* set rejection in untreated animals. The histologic appearance of the homografts in these rabbits were also impressive; here, marked hyperemia and hemorrhages were the rule and, in some of the grafts, clusters of hemocytoblasts were seen. These hemocytoblasts may have been introduced into the graft as a consequence of the hyperemia and hemorrhage. Czitober and Gollerkeri have recently demonstrated the presence of hemocytoblasts in the peripheral blood of antigenically stimulated rabbits.[21]

The reason for this remarkable "overshoot" is poorly understood, although it is evident from Groups O, N, and P that the reaction is at its maximum in the simultaneous presence of the graft and 6-MP. Group N demonstrated that the "overshoot" reaction slowly subsided after 6-MP was discontinued; however, even though the graft was in a far advanced state of destruction, the continued administration of 6-MP, as in group O, resulted in persistent lymphoid hyperplasia with the additional features of hyperemia and hemorrhage. This picture might be explained on the basis of small amounts of antigen which persisted in the graft, even though it appeared rejected, plus the toxic effects of 6-MP on the lymph nodes. The results of group P suggest a "priming" action, for when the second graft was applied (without further 6-MP treatment) the reaction provoked was more pronounced than that seen at the peak of a second set reaction in untreated animals. Perhaps some of the cells stimulated to proliferate by the combined actions of the homograft and 6-MP were reactivated by the second graft. The observation that continuous antigenic stimulation combined with continuous 6-MP administration (as in Groups Q and R) provoked responses comparable to those seen in group H might indicate the limits of the "overshoot."

The results of these experiments strongly suggest the development of 6-MP resistance by antigenically stimulated lymphoid cells. These "resistant" cells, presumably the result of a random mutation, might have been selected by two pressures: (1) antigen, which served as the initial growth stimulus, and (2) 6-MP, which eliminated "sensitive" hemocytoblasts and allowed the resistant cells to grow in an unrestrained manner. According to this hypothesis, those rabbits whose immune mechanisms were unaffected by 6-MP had a rapidly evolving colony of 6-MP resistant lymphoid cells, while those in which 6-MP

had a marked effect in suppressing transplantation immunity had a slow rate of selection of drug resistant cells. Thus it may be speculated that the problem of the inhibition of the homograft reaction has many points in common with the therapy of another proliferative condition, acute leukemia. The relationships between immune responses and leukemia have been discussed previously and attention was drawn to certain similarities between them.[22] Regardless of interpretation, the finding of a state resembling 6-MP "resistance" in lymphoid cells suggests several lines of experimentation, including the possibility of isolating "clones" of antibody-forming cells, the use of a biochemical marker of antibody cells and the study of antimetabolite resistance in nonmalignant tissues. These results may also indicate additional approaches to the chemical "treatment" of homograft immunity. Ehrlich, who first recognized the phenomenon of drug resistance in microorganisms,[23] advocated two approaches to its solution: (1) delivery of the maximum amount of drug to the microbe in the shortest time and (2) combined therapy by agents which attack the organism in different ways. The homograft reaction may represent a system to which these principles could be applied.

Summary

The effects of 6-mercaptopurine and thioguanine on the morphologic responses of lymph nodes and spleen were studied in the rabbit. The main effect of these drugs was to inhibit the proliferation of hemocytoblasts, cells considered to be immunologically competent. Homograft rejection did not occur as long as these cells were suppressed. Rabbits which rejected their grafts in spite of continued 6-mercaptopurine treatment developed large numbers of hemocytoblasts in their lymphoid apparatus. These cells were thought to be drug resistant and analogous to resistant leukemic cells.

Summario in Interlingua

Le effectos de 6-mercaptopurina e de thioguanina super le responsas morphologic del nodos lymphatic e del splen esseva studiate in le conilio. Le major effecto de iste drogas esseva que illos inhibiva le proliferation del hemocytoblastos, le quales es considerate como immunologicamente competente. Le rejection de homograffos non occurreva si longo que ille cellulas esseva supprimite. Conilios que rejiceva lor graffos in despecto de un continue tractamento a 6-mercaptopurina disveloppava grande numeros de hemocytoblastos in lor apparato lymphoide. Esseva opinate que iste cellulas esseva pharmaco-resistente per analogia con resistente cellulas leucemic.

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Janine A. André, M.D., Research Fellow, Blood Research Laboratory, New England Center Hospital; Assistant in Medicine, Tufts University Scrool of Medicine, Boston, Mass. Formerly Attacheé de Recherche au Centre Nationale de la Recherche Scientifique, Service du Professeur Gadrat, Pourpon, Toulouse, France.

Robert S. Schwartz, M.D., Research Associate, Blood Research Laboratory, New England Center Hospital, and Assistant Professor of Medicine, Tufts University School of Medicine, Boston, Mass.

W. J. Mitus, M.D., Research Associate, Blood Research Laboratory, New England Center Hospital, and Assistant Professor of Medicine, Tufts University School of Medicine, Boston, Mass.

William Dameshek, M.D., Director, Blood Research Laboratory, New England Center Hospital, and Professor of Medicine, Tufts University School of Medicine, Boston, Mass.