RESISTANCE OF LONG-LIVED LYMPHOCYTES AND PLASMA CELLS IN RAT LYMPH NODES TO TREATMENT WITH PREDNISONE, CYCLOPHOSPHAMIDE, 6-MERCAPTOPURINE, AND ACTINOMYCIN D*

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Many drugs are known which can alter or suppress immunological reactions (1, 2). These are of great current interest because of the need to find methods to prevent rejection of organ homografts and to control autoimmune diseases in humans. Among the drugs used most frequently at the current time are prednisone, cyclophosphamide, 6-mercaptopurine (or its analogue, azathioprine), and actinomycin D (dactinomycin) (3). All of these drugs are immunosuppressants in laboratory animals, but, in general, they are less effective in altering anamnestic responses than primary responses, and they have little effect on established antibody production (1). Long-lived lymphocytes are probably responsible for immunological memory (4), i.e., the capability for an anamnestic response, and long-lived plasma cells are, at least in part, responsible for persistent antibody production (5). We have recently shown that these long-lived cells are relatively resistant to X-radiation (6). The experiments described in this paper were designed to study the effects of the representative immunosuppressant drugs mentioned above on long-lived cells in rat lymph nodes.

Materials and Methods

Animals.—Two-month-old female Sprague-Dawley rats bred at this Laboratory and fed on Purina Laboratory Chow and tap water ad libitum were used. They were kept in groups of five in suspended wire cages.

Immunization.—To ensure a large number of persisting plasma cells, a secondary immunological stimulus was given before the tritiated thymidine (5). Each rat received 0.10 ml of Typhoid-Paratyphoid A and B vaccine (TAB vaccine), Wyeth Laboratories, Marietta, Pa.,

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No. 87338, subcutaneously in each hind foot pad with an interval of 4 weeks between the two injections.

Tritiated Thymidine Labeling.—Tritiated thymidine, New England Nuclear Corp., Boston. Mass., Lot No. 209-111, 6.7 c/mmole, was injected subcutaneously into the hind foot pads at 16, 24, 40, 48, 64, 72, 88, and 96 hr after the second injection of TAB vaccine in doses of 5 μc in 0.1 ml in each foot pad at each injection. At this time the rats weighed between 200 and 250 g, so the total dose (80 μc) received in the 4 days was between 0.32 and 0.40 μc/g of rat weight.

Drugs.—All the drugs were injected intraperitoneally daily, starting 31 days after the last dose of tritiated thymidine. One group of rats received no drug and served as controls. Four other groups were injected with one of each of the drugs studied. Prednisone, USP, Robinson Laboratory, San Francisco, Calif., No. 802121, was suspended in saline and was injected initially at a dose of 1.25 mg/day. Cyclophosphamide, "Cytoxan," Meade Johnson, Evansville, Ind., No. MBJ54, was dissolved in saline and was started at a dose of 0.25 mg/day. Actinomycin D, "Lyovac Cosmegen," Merck Sharp and Dohme, West Point, Pa., No. 12701, was dissolved in water and started at a dose of 1.25 μg/day. 6-Mercaptopurine, "Purinethal," Burroughs Wellcome, Tuckahoe, N. Y., No. 47031, was suspended in saline and dissolved by adding the minimum necessary amount of 1.0 N NaOH, and was started at a dose of 0.5 mg/day. The dose of each drug was doubled weekly for 5 weeks except in the case of prednisone, which was not given in doses greater than 5 mg daily (Figs. 2–5). The drug solutions or suspensions were made twice a week and were stored frozen. Further courses of 6-mercaptopurine and of actinomycin D were given to new groups of tritiated thymidine–labeled rats starting at doses of 8 mg/day and 20 μg/day, respectively, again doubling the doses on successive weeks until the rats died of drug toxicity.

Preparations of Radioautographs.—Pairs of rats from the control group were killed 3, 17, and 31 days after the last dose of tritiated thymidine, and on each occasion on which drug-treated animals were killed. Pairs of rats from each group receiving drugs were killed, usually at weekly intervals after the start of drug injections. In addition, pairs of rats receiving 6-mercaptopurine and actinomycin D were killed 3 days after the start of drug injections.

One-half of each popliteal node from each rat was teased individually into a small drop of calf serum and the cells were smeared onto gelatin-coated slides. The smears were fixed in 89% methanol containing 1% acetic acid for 30 min, washed, and prepared as radioautographs using Kodak AR 10 stripping film. The radioautographs were developed and stained with Giemsa after exposure for 4 wk at 4°C.

Tissue from spleen, thymus, a piece of intestine including a Peyer's Patch, a mesenteric lymph node, an aortic lymph node, and the remaining halves of each popliteal node from each rat were fixed in 65% ethanol–5% acetic acid–5% formalin, paraffin-embedded, cut at 4 μ, and processed for radioautography using Kodak NTB 3 emulsion diluted 1:1 with distilled water. After 2 wk exposure at 4°C, the radioautographs were developed and stained with methyl green–pyronin.

Animals dying during the course of the experiment, presumably from drug toxicity, were examined grossly, but in each case it was felt that the tissues were too autolyzed for use for microscopy or radioautography.

Observations.—The popliteal node smears of the rats were counted individually for 1000 cell differential, classifying cells as plasma cells, small lymphocytes, medium cells, and blast cells as previously described (5). The per cent of each cell type with three or more grains over the nucleus was determined by counting 1000 small lymphocytes and as many of each of the other cell types as could be found, usually 50, never less than 25 except in some of the most severely hypoplastic nodes from prednisone- or cyclophosphamide-treated rats. The individual grain counts of 50 labeled small lymphocytes were used to calculate the mean grain count for

each popliteal node. The mean grain counts of the other cell types were determined by using all of the cells of that type found on the four smears of each group of rats at each time point.

The sections of the various lymphoid organs were examined for the histological features of each organ, and for the location and nature of labeled cells. Particular attention was paid to (*a*) the number, size, and mitotic activity of germinal centers; (*b*) the thickness and cellular content of diffuse cortical tissue, including the periarterial lymphocyte cuffs of the spleen; and (*c*) to the thickness and cellular content of medullary cords or of the plasma cell content of spleen and intestinal submucosa.

Antibody Titrations.—Blood obtained from each rat at death was allowed to clot, and the serum was stored frozen. Sera were inactivated at 56°C for 30 min before use. Agglutinin titers were determined by serial twofold dilutions read under a dissecting microscope after 1 hr at room temperature and 18 hr at 4°C. Titers were determined for each serum against both saline-washed TAB vaccine and against an ethanol-killed suspension of *Salmonella derby*, American Type Culture Collection, No. 6960, an organism sharing "O" but not "H" antigens with TAB vaccine.

RESULTS

Controls.—The popliteal nodes, the aortic nodes, and the spleens of the control rats killed 3 days after the last dose of tritiated thymidine, 7 days after the secondary stimulus, had hyperplastic germinal centers with many mitotic figures, hyperplastic cortical tissue with many large pyrininophilic cells, and hyperplastic medullary cords filled with typical plasma cells of varied maturity. Thereafter smaller germinal centers with less mitotic activity persisted in the popliteal nodes, and the cortices and medullary cords thinned to a size which was maintained throughout the rest of the experiment. Slightly larger germinal centers persisted in the aortic nodes and spleens. The mesenteric nodes and Peyer's patches never changed in appearance. The Peyer's patches consistently had the largest, most active germinal centers of any of the lymphoid organs studied. A summary of the histological findings is shown in Table I.

The individual values for the logarithms of the per cent of small lymphocytes labeled in the popiteal nodes of the control rats are shown in Fig. 1. A regression line (method of least squares) and the 99% confidence limits for the logarithms of individual points around the regression line for the data from the rats killed 31 or more days after the last dose of tritiated thymidine are also shown. The data from the rats killed earlier than 31 days after the last dose of tritiated thymidine are not included in the regression line or the 99% confidence limits because prior work (5) had indicated that the rate of disappearance of labeled cells was more rapid before than after 30 days. Table II shows that from 17 days following the course of tritiated thymidine, the mean grain counts of the labeled small lymphocytes remained constant, indicating that a stable population of cells was being studied.

Labeled mature plasma cells were seen in the sections of all the popiteal nodes of the control rats. On the radioautographs of smears prepared from the popliteal nodes the per cent of plasma cells with persistent label varied from a mean of

TABLE I

Summary of Histological Findings in Control and Drug Treated Rats

Days after last dose of tritiated thymidine	Days of drug treatment (cumulative)	Histology*	Controls	Prednisone	Cyclophosphamide	6-Mercaptopurine	Actinomycin D
3		Germinal centers	++++				
		Diffuse cortices	++++				
		Medullary cords	++++				
17		Germinal centers	++++				
		Diffuse cortices	+++				
		Medullary cords	+++				
31		Germinal centers	+++				
		Diffuse cortices	+++				
		Medullary cords	++				
34	3	Germinal centers	+++			+++	+++
		Diffuse cortices	+++			+++	+++
		Medullary cords	+++			++	+++
38	7	Germinal centers	+++	+++	++	++	+++
		Diffuse cortices	+++	+++	+++	+++	+++
		Medullary cords	+++	++++	+++	++	++
45	14	Germinal centers	+++	++	++	+++	+++
		Diffuse cortices	+++	+++	+++	+++	+++
		Medullary cords	+++	+++	+++	+++	+++
52	21	Germinal centers	++	+‡	+	+++	+++
		Diffuse cortices	+++	+	+++	+++	+++
		Medullary cords	+++	+++	+++	+++	++
59	28	Germinal centers	+++	+‡	+	++	+++
		Diffuse cortices	+++	++	++	+++§	+++
		Medullary cords	+++	++	+++	++	+++
66	35	Germinal centers		+‡	0		
		Diffuse cortices		+	+		
		Medullary cords		++	+++		
69	38	Germinal centers	+++			++	+++
		Diffuse cortices	+++			+++‖	+++
		Medullary cords	+++			++	+++

Second Course

Days after last dose of tritiated thymidine	Days of drug treatment (cumulative)	Histology	Controls	Prednisone	Cyclophosphamide	6-Mercaptopurine	Actinomycin D
38	7	Germinal centers	+++			+++	++
		Diffuse cortices	+++			+++§	++‖
		Medullary cords	+++			+++	+++
45	14	Germinal centers	+++			+‡	
		Diffuse cortices	+++			+++‖	
		Medullary cords	+++			+++	

* ++++ = hyperplastic, +++ = "normal," ++ = moderate hypoplasia, + = severe hypoplasia, and 0 = total absence. See text and Figs. 2–5 for dose levels at different times.

‡ Germinal centers present only in Peyer's patches.

§ Thymic cortex pyrinophilic.

‖ Thymic cortex severely hypoplastic.

93% with a mean grain count of 19 at 3 days after the last dose of tritiated thymidine, to a mean of 8% with a mean grain count of 23 at 69 days after the end of the course of thymidine. The individual values for both the per cent of plasma cells labeled and the mean grain counts of the labeled plasma cells varied at the intermediate time points, but, in general, showed the biphasic rate of decrease in numbers and the variable but persistently high grain counts seen in earlier experiments (5, 6). These data are not presented in detail because we no longer believe that the quantitative data obtained from labeled plasma cells on radioautographs of smears of lymph nodes are truly representative of the intact nodes. Radioautographs of *sections* consistently show a higher per cent of persisting labeled plasma cells than do radioautographs of *smears* from the same node. This is due to the fact that the older labeled plasma cells persist in the distal, most fibrous portions of the medullary cords, and in the hilar tissues, and are not as readily teased out of the node as are lymphocytes of the cortex, or as are the younger plasma cells in the more proximal portions of the medullary cords.

Labeled medium cells and blast cells were found on smears of all the control rats, but the numbers were small and the mean grain counts did not exceed 4.0 after the first 17 days following the end of the course of tritiated thymidine. The data were similar to those given in detail previously (5).

Effect of Prednisone.—Those rats receiving injections of prednisone daily appeared normal until they had received a dose of 5 mg/day for 5 days. At that time their average weight was 186 g compared to the control average of 228 g.

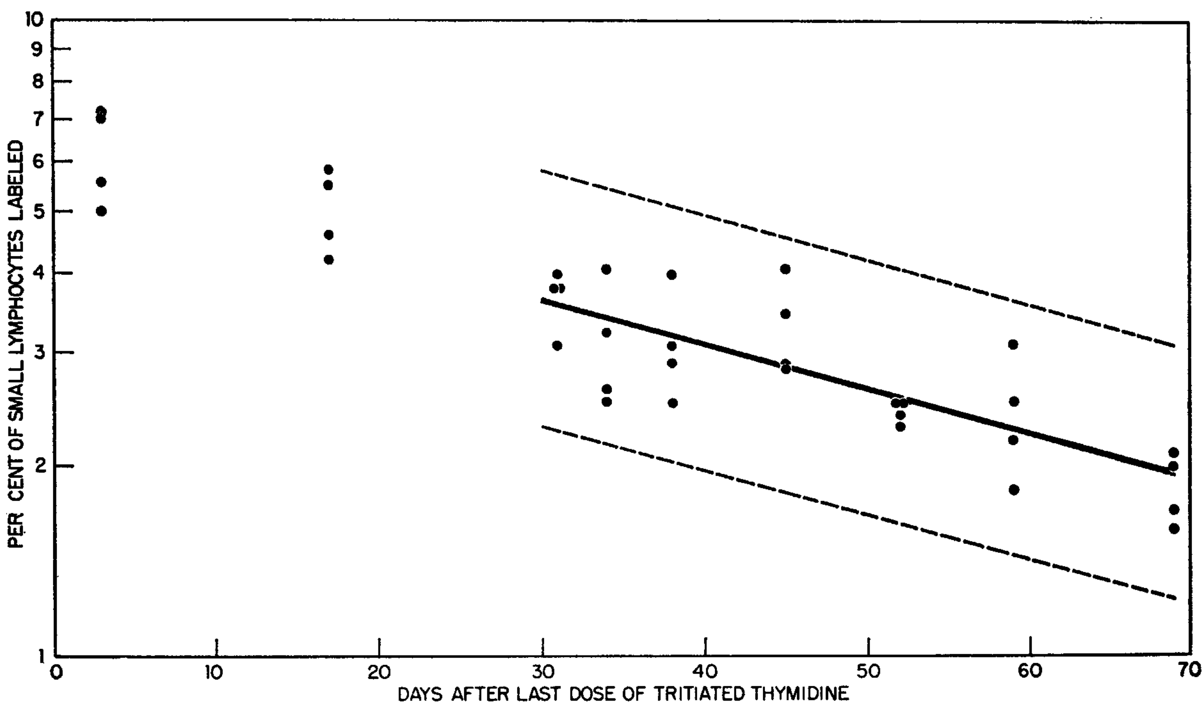

FIG. 1. Graph showing the logarithms of the individual values for per cent of small lymphocytes labeled in each popliteal node of the untreated, control rats following the course of injections of tritiated thymidine. The regression line (———) and the 99% confidence limits (------------) for the logarithms of individual values around the regression line for the data from rats killed 31 or more days after the last dose of tritiated thymidine are shown.

One rat died. This dose of prednisone was continued for 14 more days without further increase. The surviving rats lost no more weight and were free of gross infection at death. Marked hypoplasia of the diffuse cortical tissue of all the lymphoid organs was found in rats killed after 7 days of treatment with 5 mg prednisone daily. Germinal centers persisted only in Peyer's patches. Medullary cords and plasma cells appeared unaffected after 7 days at this dose, but after 14 days there was a decrease in size of the medullary cords.

The logarithms of the individual values for per cent of persisting labeled small

TABLE II

Means of Mean Grain Counts of Labeled Small Lymphocytes in the Popliteal Nodes of Control and Drug-Treated Rats

Days after last dose of tritiated thymidine	Days of drug treatment (cumulative)	Control rats	Prednisone-treated rats	Cyclophosphamide-treated rats	6-Mercaptopurine-treated rats	Actinomycin D-treated rats
3		11.2				
17		8.1				
31		8.3				
34	3	6.9			7.8	7.3
38	7	7.2	7.8	6.7	7.0	6.9
45	14	7.3	6.1	5.7	8.5	7.4
52	21	7.1	9.7	5.9	7.2	6.5
59	28	7.2	8.0	7.8	6.9	7.2
66	35		8.8	7.9		
69	38	7.6			7.8	7.1
Second Course						
38	7	7.2			5.8	6.7
45	14	7.3			7.5	

lymphocytes in the popliteal nodes of the rats receiving daily injections of prednisone are shown in Fig. 2, along with the regression line and 99% confidence limits calculated from the control data. At the time lymphoid hypoplasia was first apparent, after 7 days of 5 mg prednisone daily, the per cent of small lymphocytes labeled was still the same as that of the control rats. However, as the prednisone injections were continued, the per cent of labeled lymphocytes increased progressively, eventually exceeding the 99% confidence limits of the control data. The mean grain counts of the persisting labeled small lymphocytes remained similar to those of the controls, Table II.

Persistently labeled plasma cells were found in sections of the popliteal nodes of all prednisone-treated rats, but, after prolonged treatment, they decreased in number in parallel with the decrease in total number of plasma cells. The few labeled plasma cells found on the radioautographs of the smears of these pop-

liteal nodes had grain counts within the range of the controls. The percent of labeled medium cells and blast cells, and their mean grain counts, remained small and were comparable to those of the controls.

Effect of Cyclophosphamide.—Rats receiving cyclophosphamide appeared

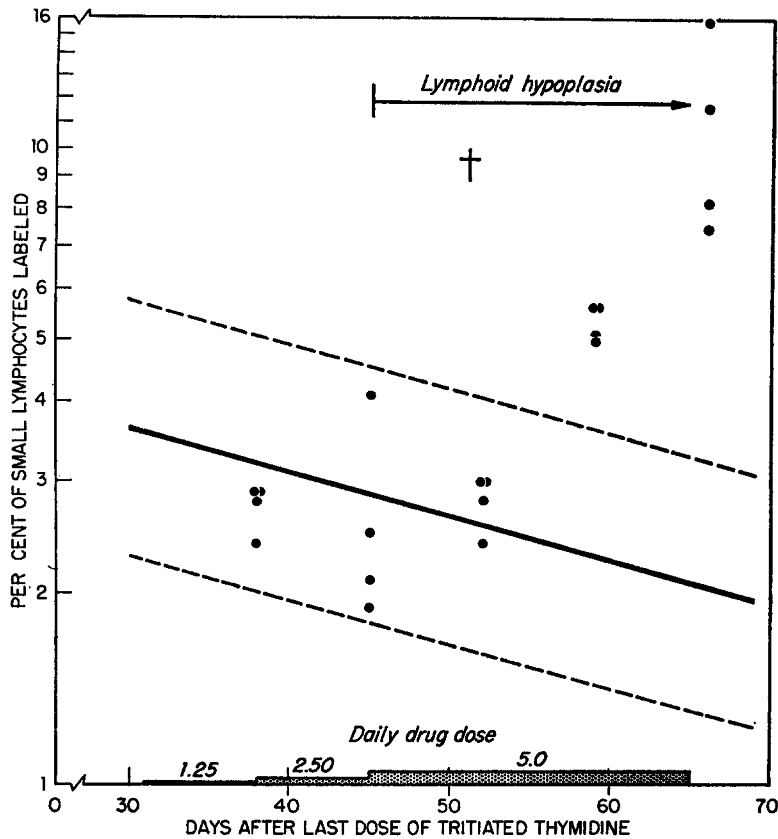

FIG. 2. Graph showing the logarithms of the individual values for per cent of small lymphocytes labeled in each popliteal lymph node of the rats receiving prednisone compared to the regression line (————) and the 99% confidence limits (-----------) calculated from the control data. The daily intraperitoneal drug dose is shown at the bottom. The time of death of the treated rat is indicated (†), as is the most significant pathological finding.

normal until they had reached a dose level of 4.0 mg daily, at which time they developed alopecia and their weight fell below the controls' average of 228 g to an average of 205 g. Rats receiving only 0.25 mg of cyclophosphamide daily had smaller germinal centers than the control rats. Hypoplasia of the diffuse lymphoid tissues was not seen until a dose of 2.0 mg daily had been given for 7 days. Germinal centers were completely absent after 7 days at a dose of 4.0

mg. daily. Medullary cords and plasma cells did not appear to be affected at any dose studied, and an increase in per cent of plasma cells in the differential counts of the smears of the popiteal nodes was found after lymphoid hypoplasia was noted histologically.

The logarithms of the individual values for per cent of small lymphocytes labeled in the popliteal nodes of the cyclophosphamide-treated rats are shown in Fig. 3. At the lower doses, 0.25 through 1.0 mg daily, the per cent of small

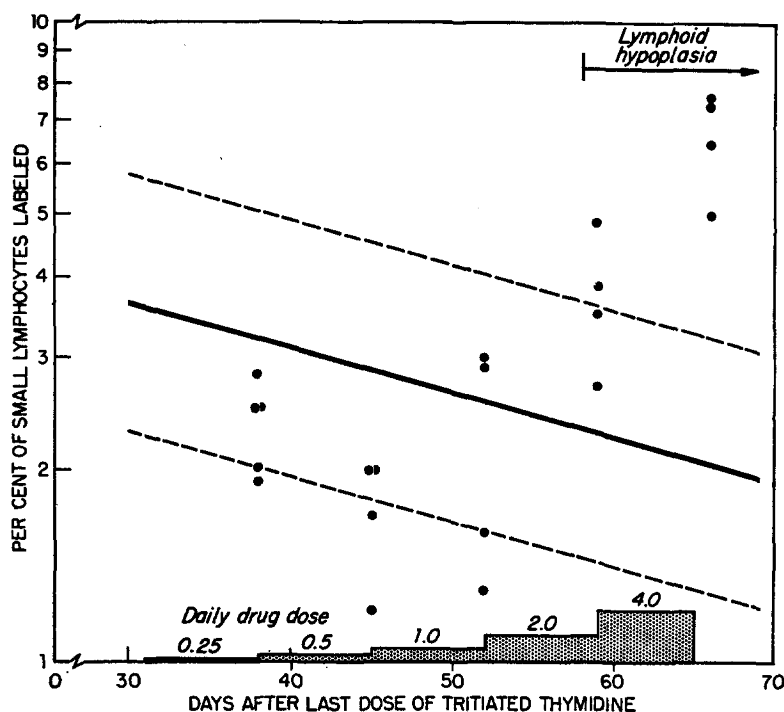

FIG. 3. Graph showing the data described in Fig. 2 for the rats receiving cyclophosphamide.

lymphocytes labeled tended to be less than in control rats, but at the dose producing lymphoid hypoplasia, 2.0 mg daily, the per cent of lymphocytes labeled became greater than in control rats, and, after 7 days treatment of 4.0 mg daily, the per cent of lymphocytes labeled exceeded the 99% confidence limits of control data. The mean grain counts of the persistently labeled small lymphocytes remained similar to those of the controls, Table II.

The mean of the per cent of plasma cells labeled on the smears of the popliteal nodes of the rats which had received 4.0 mg of cyclophosphamide daily for 7 days was 21%, more than twice the value for any of the other groups of rats this long after the last dose of tritiated thymidine. The mean grain counts of

these plasma cells remained within the range of those of the control rats. The per cent of medium cells and of blast cells labeled, and the mean grain counts, were comparable to the control data throughout, although fewer of these cells were found in the differential counts of the popliteal node smears from cyclophosphamide-treated rats than in control rats.

Effect of 6 Mercaptopurine.—In contrast to those rats receiving prednisone or cyclophosphamide, those receiving 6-mercaptopurine died before marked effects on lymphoid tissue were evident. An initial group of 12 rats were started on a dose of 0.5 mg daily, which was increased progressively to 8.0 mg daily. One rat died after receiving 4.0 mg for 7 days and another died after receiving 8.0 mg for 3 days, both having bled into their small intestine. The surviving rats at these dose levels had nearly normal appearing lymphoid tissue. The thymic cortex, germinal centers, and medullary cords were slightly smaller than in control rats. The diffuse cortical lymphoid tissues were unaffected. A second group of rats was started on a course of progressively increasing doses starting at 8.0 mg daily. After 7 days, the only change found in the lymphoid tissues was hypoplasia of the thymic cortex with replacement of the small thymocytes with large pyrininophilic cells. One rat killed after 7 days of 16 mg 6-mercaptopurine daily had hypoplasia of the thymic cortex and no germinal centers except in Peyer's patches, but still had normal diffuse lymphoid tissues and medullary cords. When the dose was increased to 32 mg daily, the remaining rats died with widespread scattered hemorrhage within 5 days.

Neither the per cent of small lymphocytes labeled, Fig. 4, nor the mean grain counts of the labeled small lymphocytes, Table II, varied significantly from the control values. Labeled plasma cells persisted in the medullary cords of the popliteal nodes and were found on the smears of these nodes with a similar frequency and with similar grain counts as the control values.

Effect of Actinomycin D.—During the first 5 wk course of progressively increasing doses of actinomycin D (from 1.25 μg to 20 μg per day) no gross or histological changes were noted in lymphoid tissues. All rats developed serosanguinous ascites, edema, and focal hemorrhages at dose levels of 10 μg or more daily. A second group of rats were started on initial doses of 20 μg actinomycin D daily. After 7 days thymic cortical hypoplasia was present, and one of two rats killed at this time had hypoplastic diffuse cortical tissue. The remaining rats of this group were then given 40 μg daily, but all died over a weekend and were inadvertently discarded before being examined.

The logarithms of the values of per cent of small lymphocytes labeled in the popliteal nodes of the actinomycin D-treated rats remained within the 99% confidence limits of the control data except for the one rat with lymphoid hypoplasia, whose nodes had an increased per cent of labeled lymphocytes, Fig. 5.

Throughout the experiment, labeled plasma cells, medium cells, and blast cells were found on radioautographs of sections and of smears of the popliteal

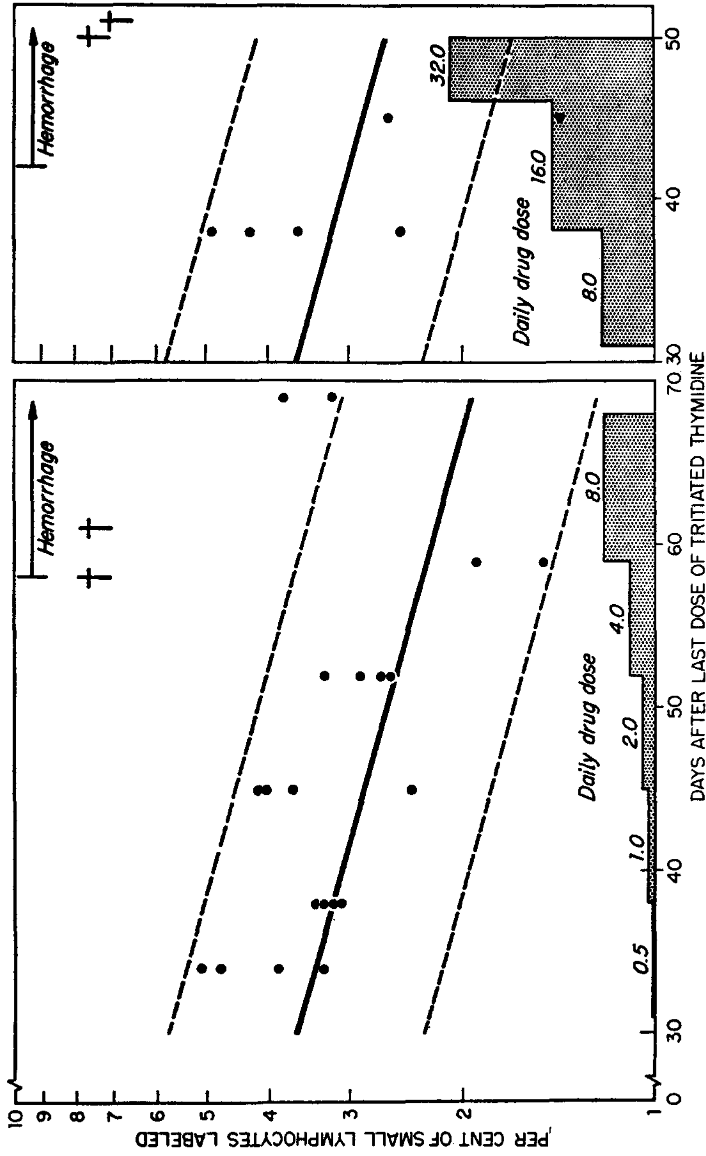

FIG. 4. Graph showing the data described in Fig. 2 for the rats receiving 6-mercaptopurine.

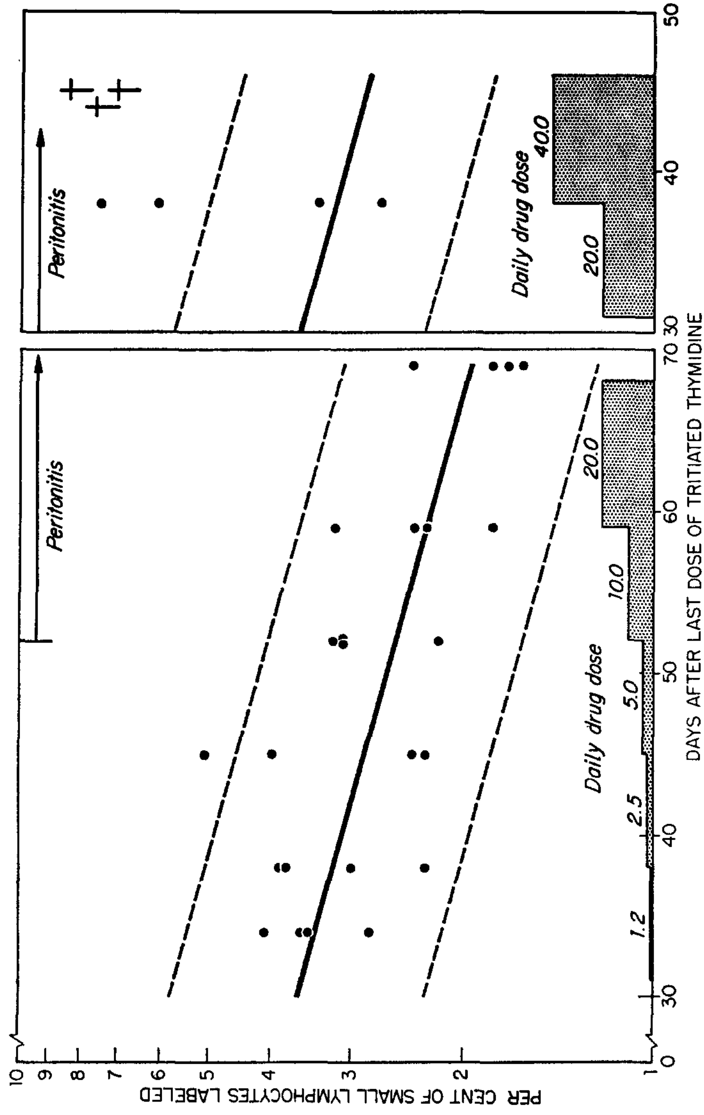

FIG. 5. Graph showing the data described in Fig. 2 for the rats receiving actinomycin D.

nodes of all actinomycin D-treated rats in the same numbers and with the same mean grain counts as in nodes from control rats.

Antibody Titers.—At no time was there more than a two-tube difference between the means of the titers against TAB vaccine ("H" agglutinins) of the control rats and of the rats treated with any of the drugs. This was also true of the titers against *S. derby* ("O" agglutinins), except for one pair of prednisone-treated rats and one pair of cyclophosphamide-treated rats, each having had prolonged treatment, which had mean titers three tubes greater than the mean titers of the controls.

DISCUSSION

The data presented in this paper indicate that long-lived lymphocytes and plasma cells in rat lymph nodes survive treatment with the four immunolytic drugs, prednisone, cyclophosphamide, 6-mercaptopurine, and actinomycin D. The reason for considering the persistently labeled lymphocytes and plasma cells long-lived and not an artifact of reutilization of tritium-labeled degraded nuclear material have been given before in detail (5, 6). The principle reason is that these cells do not themselves synthesize DNA, and that their precursors, the medium cells and blast cells, are not sufficiently labeled 30 days after the course of tritiated thymidine to be forming the number of mature cells or mature cells with as high grain counts as are found.

We used daily injections of drugs to closely approximate the manner of use in humans. However, most of the prior studies of the effects of these drugs on immune processes in laboratory animals have used single doses or short courses. Thus it is difficult to find data, particularly in respect to drug doses, which can be used to compare the effects on long-lived cells noted in this study and the effects on acute immunological reactions studied by others. The doses of prednisone used in our work exceed the comparable levels of cortisone acetate (4 mg/100 g rat weight per day) reported by Berglund (7) to suppress a primary response in rats. Santos and Owens (8) found that 20 mg/kg cyclophosphamide given for 5 days increased the induction time and decreased the peak titer of red cell agglutinins in rats, a dose which compares to the highest reached in our experiments, 16-20 mg/kg per day, and one which caused marked lymphoid hypoplasia in our rats. Buskirk et al. reported essentially the same results in rats with 10 mg/kg per day (9). Stender et al. (10) stated that repeated doses of as little as 3 mg/kg cyclophosphamide can reduce anti-Brucella agglutinin titers in rats. The highest dose reached with 6-mercaptopurine prior to the uniformly lethal dose, 16 mg/day, equivalent to 64-80 mg/kg per day, compares with that found to be effective in delaying and decreasing primary immune reactions in rats, 75 mg/kg per day for 5 days (8), and with that capable of decreasing the incidence of adjuvant arthritis in rats, 75 mg/kg per day for 3 days (11); but it is greater than that required to suppress tumor rejection in

bWr rats, 12 mg/kg per day for 16 days (12). Wust et al. (13) studied the effect of single doses of actinomycin D on the primary response of female rats of comparable weight to those we used, and reported a slight delay in induction time with as little as 10 μg, although significant delay did not appear until doses of 30-50 μg were used, lethal doses when given daily in our experience. Thus it is possible that we did not reach true immunolytic doses with actinomycin D, although the cumulative effect in causing death (13) makes it likely that a cumulative immunolytic dose was probably reached in our experiments.

The increase in per cent of small lymphocytes labeled in the popliteal nodes of the prednisone-treated rats indicates that these cells are preferentially resistant to this drug. Since it is not known how prednisone destroys lymphocytes (2), the results reported here were not predictable. Prednisone was the only one of the drugs studied which appeared to have any effect on preexisting plasma cells. It is difficult to understand why there was not a concomitant decrease in antibody titer.

The increase in per cent of small lymphocytes labeled after treatment with cyclophosphamide indicates that these long-lived cells are also specifically resistant to this drug. This result was predictable since the primary effect of cyclophosphamide, at a cellular level, is to prevent successful mitosis (2), thus reasonably having little effect on long-lived, nondividing cells. The failure of cyclophosphamide to have any effect on long-lived plasma cells confirms the suggestion made by Frisch and Davies (14).

The lack of marked effect of 6-mercaptopurine on any lymphoid tissue other than thymic cortex before the death of the treated rats was unexpected. With no great change in lymphoid tissues generally, it is to be expected that the long-lived lymphocytes and plasma cells persisted in the same relative proportions as they did in control, untreated rats. If treatment could have been prolonged, an increase in per cent of labeled lymphocytes might have been found. Morphological alterations of lymphoid tissues during 6-mercaptopurine treatment have previously been found to be variable even in rabbits (15), the animal in which immune reactivity is most susceptible to 6-mercaptopurine (2). Further, it has been suggested that the effect of 6-mercaptopurine on the manifestations of immunological reactions may be a direct antiinflammatory action (16), and that its value in human clinical situations, or that of azathioprine, may be in its ability to establish tolerance (1, 17) rather than by simply stopping antibody production. Indeed, if tolerance is primarily an attribute of specifically altered cells (18), it is possible that the ability of long-lived lymphocytes to survive treatment with 6-mercaptopurine is important in the establishment and maintenance of the tolerance inducible with this drug.

In vivo studies of the effect of single doses of actinomycin D on immunological reactions have shown primarily a delay in the appearance of

antibody without a change in subsequent peak titer (13). Morphologically, in mice, this has been associated with destruction of blast cells (19). However, only one of the rats receiving daily injections of actinomycin D in our studies had significantly altered lymphoid tissue histology prior to the reaching of a uniformly lethal dose. This discrepancy may be due to species differences. Actinomycin blocks DNA-dependent RNA synthesis (20), thus stopping the production of protein needed for cell division and, presumably, for antibody globulin synthesis. Species differences in RNA synthesis in mature lymphocytes and plasma cells probably exist, with least active new RNA synthesis occurring in these cells in rats (21). The presence of a stable messenger RNA in rat plasma cells, as suggested by Mitchell (22), would explain the ability of plasma cells to survive and to continue to produce antibody despite prolonged periods of treatment with actinomycin D.

These studies point out a cellular mechanism which may be involved in the failure of immunolytic drugs to destroy established antibody production and immunological memory. Species differences in drug action (1, 2) and in lymphoid cell metabolism (21) make it dangerous to apply conclusions from this work in rats to human medicine, but it seems reasonable to suppose that long-lived, drug-resistant lymphocytes and plasma cells are present in humans undergoing homograft rejection or with autoimmune diseases. If so, curative treatment probably will require destruction of these cells, possibly making a distinction between long-lived cells carrying specific memory for immunological reactivity and those carrying memory for immunological tolerance. Further, if there is heterogeneity in the life spans of neoplastic lymphoid cells in leukemia or myeloma, a similar situation as found in these experiments with normal lymphocytes and plasma cells may explain the failure of these drugs to produce cures of these diseases. In search for more effective agents it may be well to use the kind of experiment described here as one of the screening tests.

SUMMARY

The cells of the popliteal lymph nodes of rats were labeled for 4 days after a secondary immunological stimulus. 31 days after the last dose of tritiated thymidine, groups of rats were started on courses of daily, intraperitoneal injections of prednisone, cyclophosphamide, 6-mercaptopurine, or actinomycin D. The initially low doses of these agents were doubled in successive weeks until either lymphoid hypoplasia or death occurred. Rats from each group were killed weekly, and the percentages of persisting, labeled small lymphocytes in the popliteal nodes were determined. Sections of these nodes were examined for persisting, labeled plasma cells. The per cent of lymphocytes labeled increased while the total number of lymphocytes decreased during treatment with prednisone and cyclophosphamide. Prednisone decreased the numbers of long-

lived plasma cells, but these cells were preferentially resistant to cyclophosphamide. Neither 6-mercaptopurine nor actinomycin D had an appreciable effect on lymphoid tissues histologically nor on the proportions of labeled, long-lived lymphocytes and plasma cells before causing the deaths of the rats receiving them. These results indicate that long-lived lymphocytes and plasma cells survive treatment with the immunolytic drugs studied, and that long-lived lymphocytes are specifically resistant to prednisone and cyclophosphamide. We believe these results have an application to the attempts to find drugs useful in the treatment of immunologic rejections of organ transplants, and for therapy of autoimmune diseases.

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