Effect of Tumor Cell Inoculation on Circulating Lymphocytes[1] (36238)

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Evidence indicates that immunity to tumors, similar to that induced by allotransplants, is cell mediated (1, 2) and that the role of the lymphocyte is important in that regard (3–5). While the results of a variety of *in vivo* and *in vitro* investigations relative to lymphocyte–tumor relationship have been published (6–8), to our knowledge no information is available concerning the effects of tumor transplants in isologous systems on the quantitative aspects of lymphocytes in peripheral blood. While studying the influence of antilymphocyte serum on tumor growth (9), data were obtained which indicated that such investigations were warranted. The following reports findings in that regard.

Materials and Methods. All studies employed 8–12-week-old C3HeB/FeJ female mice housed in individual cages and fed laboratory chow and tap water. White blood cell counts and differentials were made on tail vein blood from unanesthetized mice. Preliminary observations demonstrated that the trauma and blood loss from serial counts had no effect on lymphocyte values. Blood was collected from all animals between 9 and 11 AM to eliminate diurnal variations. All lymphocytes are expressed as thousands per cubic millimeter.

Tumors employed were spontaneous C3H mammary tumors maintained by transfer in C3HeB mice and used in their 10–20th transplant generation. Methylcholanthrene (MC) tumors induced by subcutaneous injection of MC in sesame oil in C3HeB mice were used in their first and second transplant generation.

For preparation of cell suspensions, tumors and kidney were finely minced in Medium 199 and gently passed through an 86 mesh nylon screen. Cell counts were made using trypan blue to determine viability. Suspensions were diluted to contain the desired number of cells in 0.1 ml for subcutaneous or 0.2 ml for intravenous inoculation. When C3H tumor or kidney cell suspensions were employed 2.5×10^4 cells were injected. With MC tumors 5×10^5 cells were injected subcutaneously and 1×10^5 cells were inoculated intravenously.

In addition to fresh tumor cells, irradiated and frozen–thawed cell preparations were used. Suspensions of C3H tumor cells were exposed to 15,000 r or were frozen by immersion in a dry ice–acetone coolant and rapidly thawed at 37°.

Mice were inoculated subcutaneously (right hind leg at the ankle) or intravenously (tail vein) with tumor cells or normal kidney cells. Such animals had either (a) received no prior injection of normal or tumor tissue, (b) were harboring a 14-day C3H or MC tumor, (c) had a previously growing (14 day) C3H tumor removed 2 weeks before the challenge injection, or (d) had been injected 2 weeks before with normal kidney. Those with growing tumors are designated as concomitant immune mice and those whose growing tumors had previously been removed are referred to as sinecomitant immune animals. That such immunity exists has been established in prior studies (10).

ALS, prepared in rabbits as previously described (9), was administered intraperitoneally 0.2 ml daily for 3 days prior to tumor plug implantation and every 3rd day during the 14 days of tumor growth. It was discon-

[1] Supported by USPH Grants CA-05949, AI-08206, HRSF-L19.

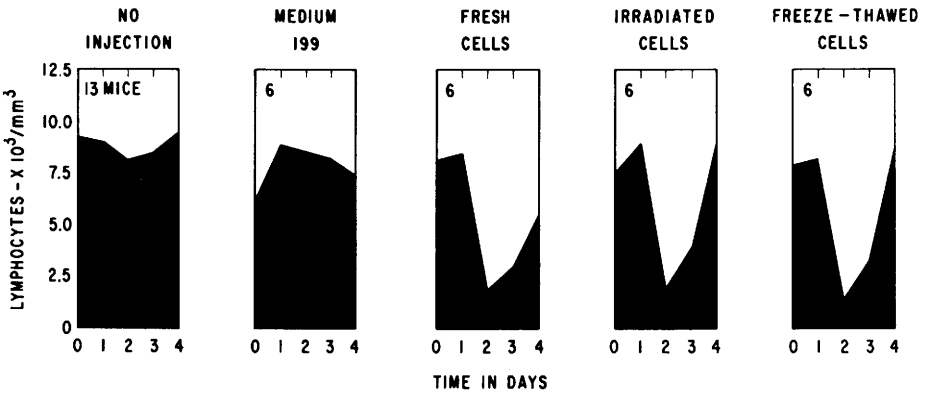

FIG. 1. Effect of subcutaneous inoculation of C3H tumor cells on circulating lymphocytes in normal animals.

tinued the day of amputation of the tumor.

Results. Effect of inoculation of C3H tumor on circulating lymphocytes. Subcutaneous inoculation of a C3H tumor cell suspension, fresh, irradiated, or frozen–thawed, produced a precipitous decrease in circulating lymphocytes (Fig. 1). A maximum depression for each group was noted the second day following injection when lymphocyte counts were less than 2,000 (18–22% of control values). Cell counts gradually returned to preinoculation levels during the next few days. Such a response was not observed in repetitive counts taken from animals receiving no injection or an inoculation of Medium 199 by itself.

Similar subcutaneous inoculation of C3H tumor cells into animals with a growing C3H tumor (concomitant immune) or into animals from which a C3H growing tumor had been removed (sinecomitant immune) failed to effect a significant decrease in circulating lymphocytes at any time during the period of observation (Fig. 2). Animals having had one or two previous injections of C3H kidney cells or having a growing MC tumor showed a decrease in circulating lymphocytes similar to that in normal animals.

Inoculation of an equivalent number of kidney cells into normal animals resulted in a similar effect on circulating lymphocytes to that observed when tumor cells were injected (Fig. 3). The magnitude of the depression, however, was slightly less (3.3 vs. 1.8). When animals were injected with kidney cells after previous exposure to tumor or to kidney cells a decrease in lymphocytes failed to occur.

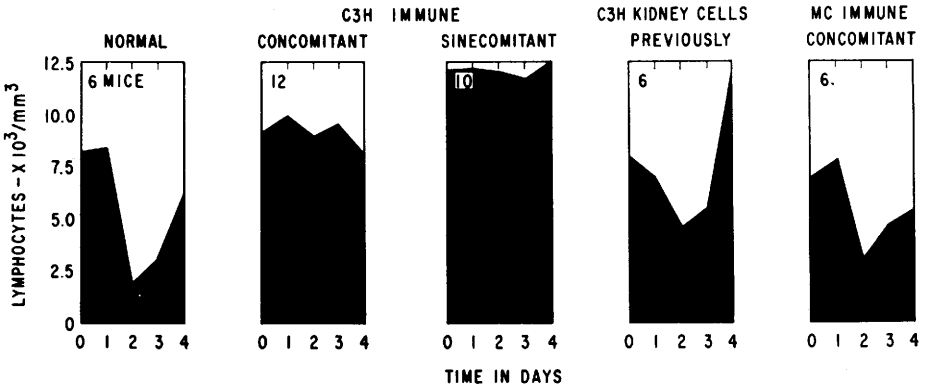

FIG. 2. Effect of subcutaneous inoculation of C3H tumor cells on circulating lymphocytes in immune animals.

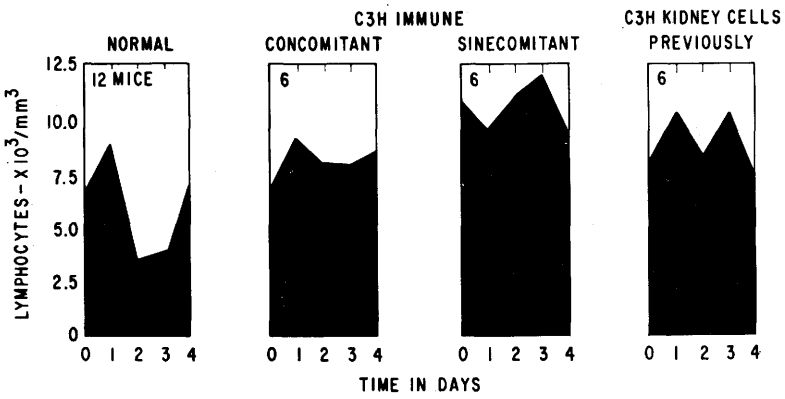

FIG. 3. Effect of intravenous inoculation of C3H kidney cells on circulating lymphocytes.

Since the lowest counts for individual animals did not invariably occur on the second day following inoculation, the mean of such values for individual animals obtained during the four-day period was recorded (Table I). Such an analysis of the data provided results in keeping with those obtained from average daily counts. Likewise of interest was the observation that initial lymphocyte counts obtained from both groups of immune animals and those having had prior kidney cell injections were significantly higher ($p < .01$) than were those in normal animals (Table II). The subcutaneous insertion of a solid plug of C3H tumor into normal mice or those previously exposed to tumor or kidney cells produced the same results as did the inoculation of a single cell suspension (Table III).

The intravenous inoculation of C3H tumor cells resulted in findings similar to those observed following the subcutaneous inoculation of tumor cells (Fig. 4 and Table I). Whereas hours after the intravenous injection of tumor cells the mean lymphocyte count in normal animals was 1.7 ± 0.3; in concomitant and sinecomitant immune animals it was 9.3 ± 2.1 and 9.9 ± 2.8, respectively. If animals had been inoculated 2 weeks prior to tumor cell injection with C3H kidney cells, a significant ($p < .01$) decrease in circulating lymphocytes was observed at 48 hr (2.7 ± 1.3).

Effect of MC-induced tumor on circulating

TABLE I. Lowest Lymphocyte Counts Recorded During 4-Day Period After Inoculation of C3H Tumor and Kidney Cells.

Inoculum[a]	Normal animals	C3H immune concomitant	C3H immune sinecomitant	MC immune concomitant	Prior kidney cell injected animals
Subcutaneous					
C3H tumor cells	1.8 ± 0.8[b] (6)[d]	6.7 ± 1.9 (12)	9.2 ± 2.2 (10)	2.9 ± 0.8 (6)	2.9 ± 2.5[c] (6)
Kidney cells	2.4 ± 0.6[c] (12)	6.9 ± 1.8 (6)	7.7 ± 0.8 (6)		5.7 ± 2.0 (6)
Intravenous					
C3H tumor cells	1.7 ± 0.3[c] (7)	7.7 ± 1.5 (6)	7.1 ± 2.4 (6)		2.7 ± 1.3[c] (12)

[a] 2.5×10^4 cells.
[b] Thousands/mm³.
[c] $p < .01$ when compared with lowest count in noninoculated groups.
[d] Number of animals.

TABLE II. Circulating Lymphocyte Counts (Preinoculation).

Immunity	No. of mice	Preinoculation counts ($\times 10^3$/mm^3)
None	65	7.2 ± 2.5
Concomitant	54	8.6 ± 2.6[a]
Sinecomitant	39	9.7 ± 2.7[a]
C3H kidney	35	9.3 ± 2.5[a]

[a] $p < .01$ when compared with nonimmune mice.

lymphocytes. The subcutaneous inoculation of normal animals with 5×10^5 cells from an MC-induced tumor resulted in a depression of circulating lymphocytes. Two days after inoculation the mean count of animals was 4.3 $\pm$ 1.5 in contrast to a preinjection count of 8.3 $\pm$ 1.8 ($p < .01$). The average lowest lymphocyte count during the 4-day period in those mice was 3.7 $\pm$ 1.0. Intravenous inoculation of such cells (1×10^5) also resulted in a depression of circulating lymphocytes, there being 4.5 $\pm$ 2.2 on the second day. The average lowest value for animals during the period of observation was 3.4 $\pm$ 1.0.

Effect of ALS on the pattern of lymphocyte response in sinecomitant immune animals. ALS was administered prior to and during the presence of immunizing C3H tumors. Following removal of tumors and cessation of ALS, peripheral lymphocyte counts were determined at 2-week intervals until they returned to pretreatment levels 8 weeks later. At that time animals were inoculated with C3H tumor cell suspensions. Peripheral lymphocytes failed to decrease; being similar to those in the non-ALS treated sinecomitant immune control group.

Discussion. Consideration of the mechanism(s) responsible for the depression of circulating lymphocytes after the initial exposure to tumor or kidney cells remains speculative. This phenomenon could possibly be related to an interference with lymphopoiesis, to a destruction of lymphocytes in the blood and lymphoid tissue, or to an alteration of normal lymphocyte recirculation between blood and lymph. It seems unlikely that the profound decreases observed are the result of a change in the rate of lymphocyte production. The long life span of most of the small lymphocytes in blood (11) would tend to preclude that explanation. That tumor and kidney cells are cytotoxic to lymphocytes so that the decrease is akin to that observed after administration of corticosteroids or antilymphocyte serum also seems unlikely. The latter agents produce their response within a few hours (12) rather than in the several days observed here. Moreover, if such were the mechanism, the injection of cells into animals which had prior exposure to such cells should have had a similar effect. More plausible would seem to be the explanation that following inoculation of such cells there occurs, as a part of the host response, an alteration of the lymphocyte recirculation. That administration of certain antigens can perturb recirculation through lymphoid tissue has been documented (13, 14). It has been suggested in this regard that the concentration of antigen in lymphoid tissue, *i.e.*, spleen and nodes, might be so great as to inhibit the flow of lymphocytes from the node into the efferent lymph (15).

Recently, it was observed (16) that following local injection of an antigen (pertussis), there was an influx of lymphocytes into the popliteal lymph node presumably from the pool of recirculating lymphocytes. It was esti-

TABLE III. Effect of Subcutaneous Implantation of C3H Tumor Plug on Circulating Lymphocytes.

Immunity	No. of mice	Daily counts ($\times 10^3$/mm^3)				Lowest count ($\times 10^3$/mm^3)
		1	2	3	4	
None	6	6.2 ± 4.1	1.7 ± 0.9	3.6 ± 1.5	5.9 ± 1.6	1.6 ± 0.8
Concomitant	6	7.4 ± 1.7	9.2 ± 1.6	10.5 ± 1.7	8.8 ± 0.9	7.2 ± 1.6
Sinecomitant	6	9.3 ± 2.7	9.1 ± 3.1	7.6 ± 1.5	8.3 ± 3.5	6.4 ± 2.0
C3H kidney	12	5.4 ± 2.4	4.6 ± 2.7	5.2 ± 2.2	7.1 ± 1.6	3.1 ± 1.0

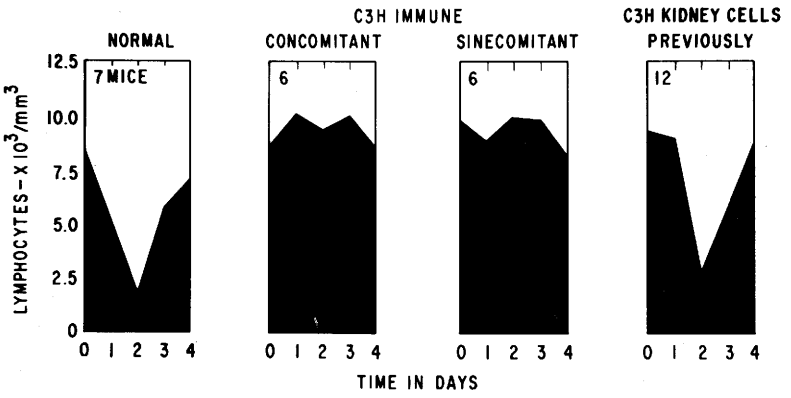

FIG. 4. Effect of intravenous inoculation of C3H tumor cells on circulating lymphocytes.

mated that about 1–2% of the circulating pool were localized in the node in 24 hr. The investigators were of the opinion that the findings could have been the result of a change in the physical structure of the node which resulted in an increase in the efficiency of the mechanical trapping of the circulating lymphocytes and also in the making of space available for the "settling down" of the circulating cells. Possibly, following injection of tumor cells, there resulted a trapping of sufficient numbers of lymphocytes to produce decreases in the circulating pool which were here observed. That this mechanism is responsible for the lymphopenia requires further proof.

Another possibility requiring consideration is that inoculated tumor cells attract to their vicinity large numbers of lymphocytes from the circulation, thus leading to a lymphocytopenia. Other studies carried out by us to evaluate the lymphoid response in tumor immunity fail to substantiate such an event (17). It was observed that tumors placed beneath renal capsules of syngenic animals evoked such a response approximately five days later in immune animals only and not to any significant extent in those which were nonimmune.

Reason for failure of a second exposure to tumor or kidney cells to produce a reduction in circulating lymphocytes is as equally uncertain as is the cause of the decrease following initial contact. The possibility may be entertained that because of the presence of committed lymphocytes in the circulation of such immune animals—as well as humoral antibody—the host response to the second exposure of such cell-bound antigens does not involve lymphoid centers. Consequently, those factors which might result in an alteration of the mechanism of lymphocyte recirculation, as suggested above, do not exist. Or alternatively, while a decrease in circulating lymphocytes may occur by such a mechanism it may be more than compensated for by proliferation of committed lymphocytes. Further investigation as to the mechanism(s) of these observations seems warranted.

Summary. When normal mice were inoculated either intravenously or subcutaneously with cells from tumors (C3H or MC) originating in isogenic hosts or with isologous kidney cells, a profound depression of peripheral blood lymphocytes occurred approximately 2 days later. A similar reduction occurred in mice injected with tumor cells when they had had prior exposure to kidney cells or to cells from a different tumor, *i.e.,* MC tumor cells prior to C3H tumor cell inoculation. No lymphocytopenia was observed, however, if tumor cells were introduced into animals possessing concomitant or sinecomitant tumor immunity, or when kidney cells were injected into mice which had previously been inoculated with syngenic kidney cells. Recipients of ALS prior to and during the presence of immunizing tumors displayed no lymphocytopenia when injected with tumor cell suspensions subsequent to a return of peripheral lymphocytes to normal levels after cessation of ALS.

The technical assistance of Francine Jencka and Christine Rudock is acknowledged with gratitude.

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Received Sept. 1, 1971. P.S.E.B.M., 1972, Vol. 139.