Hematological Changes in Tumor-Bearing Mice Treated with L-Asparaginase Active Guinea Pig Serum[1] (34393)

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Normal pooled guinea pig serum has been shown to have an antitumor effect on several animal tumors (1–4). Its action is due to the L-asparaginase enzyme (5–7). Tumor cells treated *in vitro* with guinea pig serum and incubated 6 hr at 37° retain their malignant capacity, suggesting that the effect *in vivo* may depend upon some reaction in which the animal host and guinea pig serum both participate (1). We were able to show in previous studies that L-asparaginase *in vitro* acts directly on the tumor cells and *in vivo* causes a rapid and intense host macrophage response leading to phagocytosis of tumor cells (8).

Essentially no reports concerning the effects of L-asparaginase on the hematological changes in C3H mice with the ascites form of the 6C3HED lymphosarcoma have been published. This study is an attempt to record the various changes which occur during the course of such treatment.

Materials and Methods. C3H/He Jax male mice approximately 25 g weight were used. The 6C3HED ascites lymphosarcoma was maintained in our laboratory by weekly transplants. Adult guinea pigs of mixed strain and sex were bled by cardiac puncture. The guinea pig sera were separated and stored in a freezer at −20°. The dose used to treat mice contained L-asparaginase activity of 2.0 units/ml of serum. Hematocrit, reticulocyte count, total white blood cell, differential counts, and bone marrow smears were performed on each animal. Slides for blood and ascites differential counts were stained with Wright Giemsa. Total cell counts were performed with a red blood cell pipette and a clinical hemocytometer, and hematocrits with a heparinized capillary tube in an International microcapillary centrifuge, model MD. For reticulocyte counts a methylene blue stain method was used (9).

Blood was obtained from the mice by cardiac puncture. Bone marrow was obtained from the femur; 0.5 ml of air was introduced into the proximal femoral epiphysis, pushing the medullary content through the distal epiphysis onto regular microslides. Two hundred cells were counted on each slide after staining with Wright Giemsa method. Counts were made on marrow obtained from both femurs but the difference was not significant.

Results. Twenty-seven mice were inoculated intraperitoneally (ip) with 0.2 ml containing 3×10^7 tumor cells. Previous experiments showed that this concentration of cells killed adult C3H mice in 8–12 days. Nine animals were sacrificed on each of the third, sixth, and ninth days after inoculation. An additional 12 control mice were given saline instead of tumor cells and mice were sacrificed in groups of two over 14 days. Since the blood values of the 12 control mice were similar, the mean of 12 animals is given. The results are shown in Table I.

A fall was noticed in the hematocrit from the third day to the ninth day after tumor implantation. During the same period there was a rise in reticulocytes. There was an inverse relationship between the reticulocyte count and the hematocrit values. As shown in Table I, animals with mean reticulocyte values of 0.7% had a mean hematocrit of 43.6%. As tumor growth progressed, mean reticulocyte values increased to 4.1% with concomitant drop of the mean hematocrit to 34.4%. On day 9, the mean reticulocyte level reached 6.7% and the hematocrit dropped to

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TABLE I. Blood Changes in C3H Mice with 6C3HED Lymphosarcoma.

Inoculation	No. of mice	Day sacrificed	(%) Hematocrit	(%) Reticulocyte	Leukocytes/mm³	Differential count (%) Segmented	Differential count (%) Mononuclears
Tumor cells, 3×10^6	9	3	43.6 ± 5.3	0.7 ± 0.5	5110 ± 2000	32 (12–54)[b]	68 (46–88)
	9	6	34.4 ± 5.7	4.1 ± 0.7	11,100 ± 3400	53 (22–74)	47 (26–78)
	9[a]	9	26.7 ± 1.0	6.7 ± 2.7	21,750 ± 4100	74 (68–80)	26 (20–35)
Saline controls	12	2–14	39.7 ± 4.8	2.5 ± 0.9	5400 ± 1000	35 (22–44)	65 (56–78)

[a] Two died; the values in the table were obtained from the surviving seven mice.
[b] Ranges given in ( ).

26.7%. Total white cells/mm³ rose from 5110 to 21,750 by the ninth day with an increase in relative and absolute numbers of polymorphonuclears. On the ninth day a few tumor cells were found in the blood of two mice. There was no remarkable change in the bone marrow during tumor progression.

Along with the above studies two other groups of animals inoculated ip with tumor cells were investigated to determine the effects of asparaginase on the blood picture. In the first group 6 animals were used. Three of these received 2 units each of asparaginase on the third and fifth days and were sacrificed on the sixth day. The remaining 3 received asparaginase on the third, fifth, and seventh days and were sacrificed on the ninth day. The results are presented in Table II. The overall blood picture was similar to animals of the control group treated with saline in Table I. The asparaginase-treated animals did not show the dramatic changes in the hematological picture associated with tumor progression up to the ninth day.

The second group of 12 animals with tumors were treated with asparaginase to deter-

TABLE II. Blood Changes in C3H Mice, with 6C3HED Lymphosarcoma, Treated with L-Asparaginase Active Serum.

Group	No. of mice	Day sacrificed	(%) Hematocrit	(%) Reticulocyte	Leukocytes/mm³	Differential count (%) Segmented	Differential count (%) Mononuclears
I	3	6	39 ± 4.5	4.9 ± 1.6	4200 ± 1100	39 (33–42)[a]	61 (58–66)
	3	9	37 ± 4.0	2.3 ± 2.6	2166 ± 1542	43 (42–54)	57 (46–58)
II	3	8	28 ± 4.5	11.9 ± 2.9	7900 ± 5300	69 (56–80)	31 (20–44)
	3	10	29 ± 3.4	16.7 ± 0.1	6150 ± 2460	53 (42–64)	47 (36–58)
	3	12	35 ± 5.7	5.6 ± 0.6	6100 ± 2400	52 (48–56)	48 (44–52)
	3	14	32 ± 2.3	2.3 ± 0.9	5566 ± 970	50 (44–56)	50 (38–56)

[a] Ranges given in ( ).

mine if the blood picture associated with tumor progression could be reversed. Three mice were treated on the sixth day after tumor ip and sacrificed on the eighth day. Three mice were given 2 doses of asparaginase on the sixth and eighth days and sacrificed on day 10. The remaining 6 mice were treated with 3 doses of asparaginase on days 6, 8, and 10 and 3 mice were sacrificed on day 12 and the remaining 3 on day 14. The results of this series are presented in Table II. The effects of the treatment can be seen in the blood picture of these animals. Even when assayed on day 14 the hematocrit range was 28–32%. This value, although tending toward normality, did not reach the level of the normal controls. The reticulocyte counts showed a dramatic change to normal levels. The high reticulocyte counts on days 8 and 10 were associated with the anemia which usually occurs during tumor progression. The WBC count also showed a tendency to remain normal under asparaginase therapy. These changes in the blood picture along with the rise of mononuclear cells suggest that the host's defence mechanism responded as therapy was continued.

To test for a macrophage response in tumor-bearing mice, 24 mice were given tumor ip as before. Twelve were treated with asparaginase on days 7 and 9 while the remaining 12 control mice were given normal rabbit serum which was free of asparaginase. Four animals from each group were sacrificed 20, 40, and 70 hr after the first injection of asparaginase. A differential ascites cell count was performed on three slides for each animal. Three hundred cells were counted on each slide. The results of this experiment are summarized in Fig. 1.

There was a definite increase in the number of macrophages appearing as a result of asparaginase injection whereas normal rabbit serum failed to evoke a sharp response. The number of macrophages containing ingested cells also increased over this period whereas none were seen in the control animals.

Discussion. C3H mice injected with 3×10^7 cells of the transplantable 6C3HED lymphosarcoma, ascites form, died 8–12 days after implant. During the tumor growth the animal had progressive anemia, reticulocytosis, and leukocytosis with granulocytosis. The bone marrow, however, did not show significant changes. Those mice treated with asparaginase active serum even as late as 6 days after tumor implant showed return toward normal values in hematocrit, white cell, and differential count. Reticulocyte counts varied inversely to the hematocrit; in those animals with more severe anemia a higher reticulocyte value was seen.

Study of blood changes in mice bearing tumors indicated that the hematocrit gave an easily measurable evaluation of response to treatment. In our experiments the animals were sacrificed in groups and bled by heart puncture because of the large amount of blood needed. The hematocrits, however, can be performed with a drop of blood obtained from the tail. The mechanism of the anemia was not clear. Changes in reticulocyte and leukocyte counts could also be used as an index of effectiveness of therapy. The macrophage response was a rapid index and perhaps was more sensitive than the hematocrit changes because even 20 hr after therapy had been started, a differential ascites count showed evidence of macrophage invasion.

During this period the changes in the ascites fluid in the treated animals were: (a) decrease in the total number of cells per cubic millimeter, (b) decrease in the number of tumor cells in mitosis, (c) increase in macrophages, and (d) increase in phagocytic activity (8). No phagocytic acitvity was seen in the ascitic fluid of mice treated with rabbit serum, and the changes in the blood were similar to the tumor-bearing nontreated mice.

The shifts in the white blood elements plus the rapid and intense histiocytic activity in the ascites suggest that the reticuloendothelial system plays a role in the host response in mice, with 6C3HED lymphosarcoma, treated with asparaginase active serum. This, we believe, is a nonspecific response by the host secondary to the effect of the enzyme on the tumor cells.

Summary. Animals with lymphosarcoma developed progressive anemia, reticulocytosis, and leukocytosis with granulocytosis. In

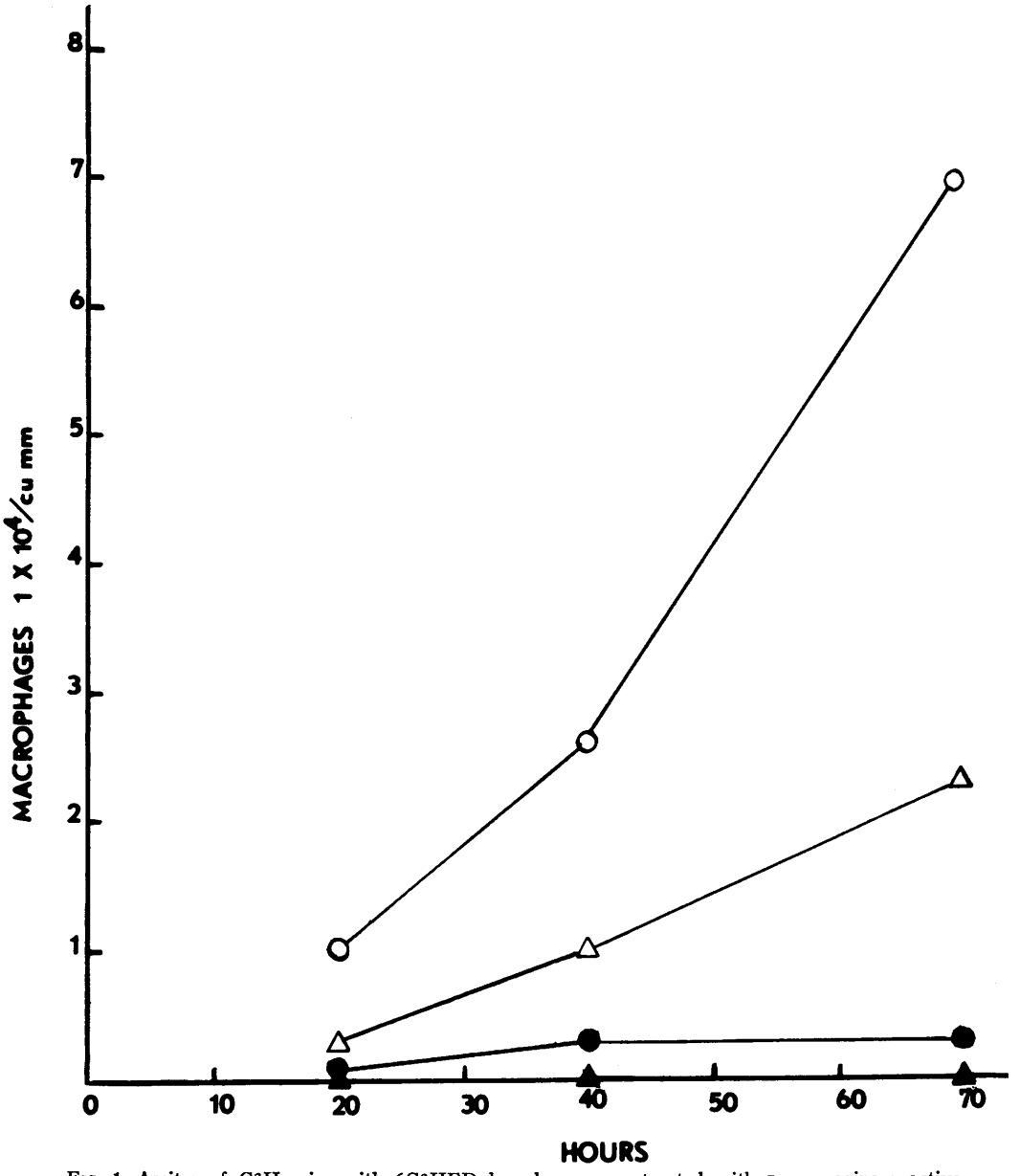

FIG. 1. Ascites of C3H mice with 6C3HED lymphosarcoma treated with L-asparaginase active serum. Number of macrophages in animals treated with L-asparaginase (○); and normal rabbit serum (●). The number of macrophages containing phagocyted cells or material in animals treated with L-asparaginase (△); and normal rabbit serum (▲).

such animals the blood returned to almost normal values after a short treatment with L-asparaginase active serum. The hematocrit was a good index of response to the serum. The ascitic fluid in mice with 6C3HED lymphosarcoma treated with rabbit serum showed no host macrophage response. Marked macrophage infiltration occurred in those mice treated with the L-asparaginase active serum.

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