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Modification of the Friend Virus Disease by Splenectomy.* (26768)

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Friend Virus infection in Ha/ICR Swiss mice produces a syndrome characterized by 2 distinct responses. The first, a malignant disorder, involves uncontrolled proliferation of reticulum cells with massive involvement of spleen and liver. The second response involves the hematopoietic system and manifests itself by an increased production of erythroblasts, erythrocytes and leukocytes. Previous studies by Mirand, *et al.*(1) demonstrated selective uptake of Fe^{59} in the spleen of infected animals and supported the hypothesis that the increased erythropoiesis was largely due to increased erythropoietic activity in the spleen. Further studies of the relationship of the spleen to the hematopoietic response were done and constitute the basis of this report.

Method. Mice employed in this study were Ha/ICR Swiss male mice 6-8 weeks old, obtained from our own breeding colonies and maintained on a diet of Derwood-Morris pellets.

Friend Virus(2) was obtained through the courtesy of Dr. Charlotte Friend, Sloan-Kettering Institute, New York City and was in its 12th serial passage in our laboratory at the beginning of this study. A pool of the virus was obtained from this passage by homogenization of 10% infected spleen mince suspension in cold Locke-Ringer solution, clarification by centrifugation for 10 minutes at 5000 G at 5°C and subsequent filtration of the supernate through a HA-grade Millipore filter with an *E. coli* marker. This filtrate was stored in sealed ampoules in a dry ice chest until used. The standard inoculum for each mouse was 0.2 ml intraperitoneally. This inoculum produced typical disease in about 85-90% of our mice. A minimum of 15 mice was used for each point in the various experimental groups.

Hematocrits were determined with Drummond microhematocrit capillary tubes. Leukocyte and erythrocyte counts were done in standard human diluting pipettes and a hemocytometer. Blood films were stained with Wright-Giemsa stain.

In the groups having splenectomies, the procedure was performed 3 days prior and 4, 19, 21 and 27 days after inoculation of the virus.

Results. Table I illustrates the characteristic splenomegaly which occurs in Ha/ICR Swiss mice following infection with Friend Virus. The rapidity with which splenic enlargement occurs is related largely to the size of the original inoculum. When a dilution of 10^{-1} was inoculated there was roughly

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TABLE I. Change of Spleen Weight in Ha/ICR Swiss Mice Inoculated with Friend Virus of Dilution Varying from 10^{-1} to 10^{-5}. Spleen weights in g as function of time after inoculation.

Days	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
0	.14	.15	.15	.14	.15
6	1.6	1.51	.17	.14	.15
12	2.0	1.12	.32	.18	.15
18	1.8	1.7	1.1	.16	.28
24	2.6	2.0	1.5	.34	.20
30	3.1	2.3	1.6	.58	.22
36	2.9	2.25	2.1	1.32	.18
42	2.2	2.1	2.2	2.2	.20

a 10-fold increase in splenic weight in 6 days (Table I). With higher dilutions more time was required for development of splenomegaly. While the increase in spleen weight occurred rapidly in the early post-infection period it soon reached a plateau (time depending on titer of inoculum) and remained relatively constant thereafter.

The leukocytosis which accompanied the splenomegaly is shown in Fig. 1 and Table II. The upper curve in Fig. 1 represents total circulating leukocyte count and Table II shows the relative numbers of granulocytes and lymphocytes. The level of leukocytes in the peripheral blood rose rapidly and reached a peak at about 20 days. Thereafter, there was a gradual decline which leveled off at lower but still significantly elevated levels. Microscopic study (Table III) of the white cells in the peripheral blood revealed essentially only immature and mature leukocytes without definitely abnormal forms of either lymphocytes or granulocytes. This might be aptly termed a "leukemoid reaction" such as may occur in response to an infectious process. Table III shows a marked granulocytosis that usually appears 26 days after infection.

One of the most sensitive indicators of Friend Virus infection was previously shown to be the development of a peripheral erythroblastosis(1,2,3). This response is shown in Table II in the line marked controls under erythroblasts.

In splenectomized mice, the marked leukocytosis following infection with Friend Virus did not occur. Table II shows that the leukocyte count was not significantly elevated as long as 48 days post-inoculation. These mice were splenectomized 3 days prior to inoculation with a 10^{-1} dilution of the virus. The controls were animals which received the same inoculum of virus but were not splenectomized. Fig. 1 shows a comparison of leukocyte levels between mice splenectomized 3 days prior to virus inoculation and mice splenectomized 4 days after virus infection. The upper curve in Fig. 1 represents the non-splenectomized controls receiving the virus and the lower lines represent splenectomized mice which were followed for a total of 60 days. No peripheral leukocytosis appeared in the splenectomized group observed for 2 months.

Fig. 2 and 3 show comparable data for the hematocrit and red blood count observed in the non-splenectomized controls whereas no rise was observed in splenectomized mice during this period. Likewise, the usual appearance of large numbers of erythroblasts in the

TABLE II. Effect of Splenectomy on Ha/ICR Swiss Mice Response to Friend Virus as Measured by Circulating Blood Cell Count. Mice splenectomized 3 days before virus inoculation. Cell counts in 10^3 per mm^3.

Days after virus inoc.	6	13	20	27	34	48
Granulocytes	5.5	1.2	4.7	2.2	8.2	3.9
Controls*	9	13	100	62	53	40
Lymphocytes	7.7	3.4	6.3	5.7	10	4.6
Controls*	17	25	115	72	50	40
Erythroblasts	0	0	0	.01	.02	0
Controls*	0	1.4	4.0	28	12	11
Liver wt (g)†	2.42	2.34	2.50	2.00	2.46	2.68

* Controls are cell counts in mice not splenectomized but which were inoculated with 10^{-1} of standard virus filtrate.

† Liver weights in mice splenectomized 3 days before virus inoculation. Avg normal liver wt is 2.40 g.

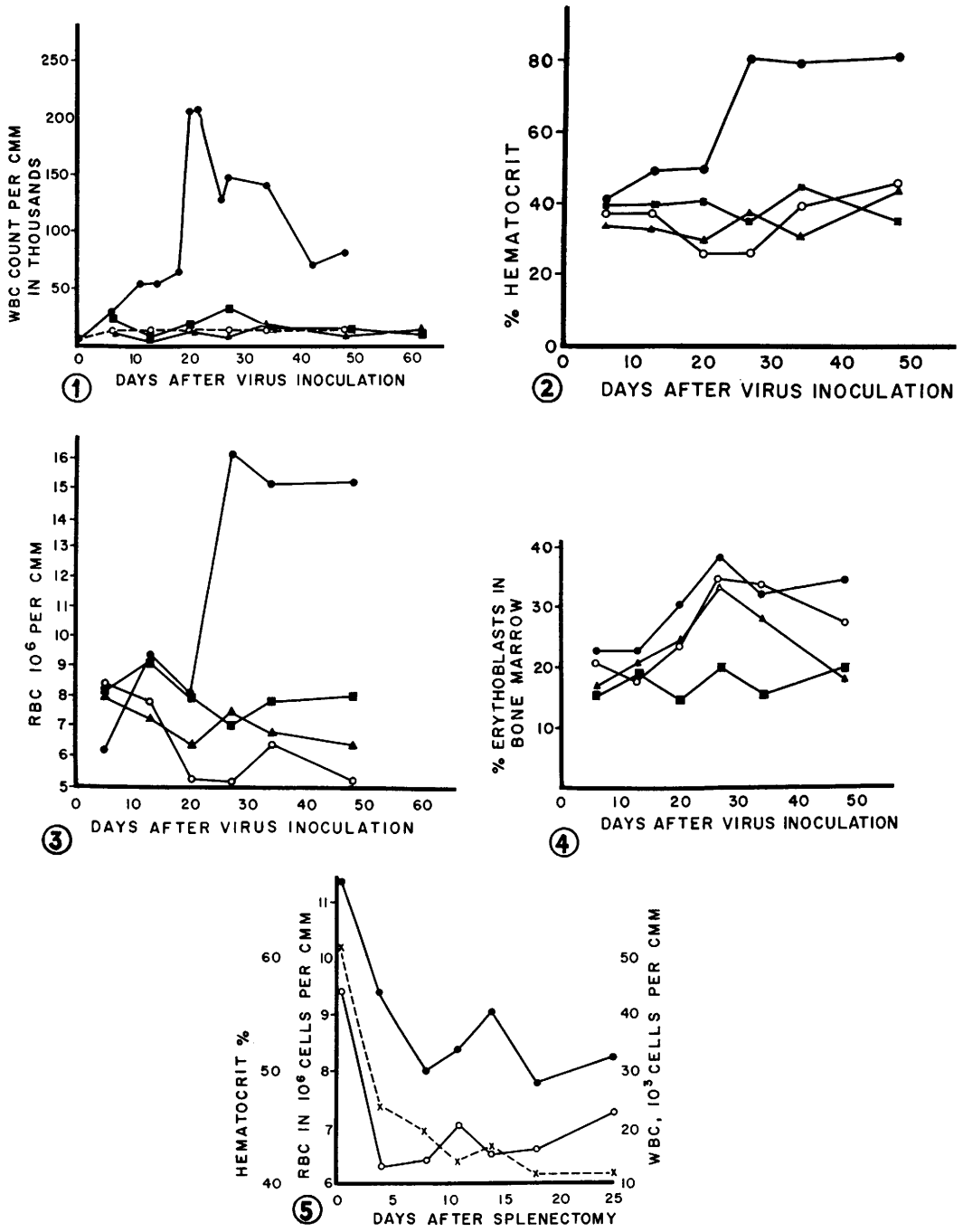

FIG. 1. Circulating total WBC count in Friend virus infected Swiss mice: —●—●—, infected non-splenectomized mice; -○- - -○-, splenectomized mice not virus infected; ▲, mice splenectomized 3 days prior to virus inoculation; ■, mice splenectomized 4 days after virus infection.

FIG. 2. Hematocrit expressed as % in Swiss mice inoculated with Friend virus plotted against time after inoculation. ●, non-splenectomized infected mice. ▲, mice splenectomized 3 days before inoculation. ○, mice splenectomized 4 days after inoculation. ■, control mice splenectomized but without virus inoculation. All inoculations at 10^{-1} standard filtrate.

TABLE III. Representative Counts of Blood of Ha/ICR Swiss Mice* Receiving Friend Virus (10^{-1}).

Days	HT	Total WBC count	Neutrophils M	J	ST	Seg	Lymphocytes Blast	Ma-ture	Mono-cytes	Other abnormal cells	Wt Spleen	Wt Liver
0	42	7,550			1	26	2	69	2	1 Ferrata	.10	2.10
6	39	19,400			4	26	1	68	1		.76	2.39
13	47	34,850		1	3	33	5	50	5	2 Megakarocytes	1.13	2.46
20	52	109,000	1	3	7	36	1	44	8	3 Friend, 1 Rieder	1.70	2.87
26	81	60,000	4	5	12	26	7	40	6	2 Friend	3.60	2.79
34	74	70,070	2	4	9	46	5	31	3	4 Friend	2.12	2.83
48	83	46,000	3	8	11	36	5	29	8	2 Ferrata, 1 Rieder	1.66	2.89
62	70	274,000	2	4	10	59	3	19	3	3 Friend	2.46	2.73

* Non-splenectomized Swiss mice.

peripheral blood did not occur in the splenectomized group. It is interesting, however, that examination of the bone marrow of the infected splenectomized group showed some increase in numbers of erythroblasts in the bone marrow (Fig. 4). However, these did not appear in the peripheral blood and there was no increase in peripheral erythrocyte levels.

This interesting relationship of the spleen to development of leukocytosis, erythroblastosis and erythrocytosis prompted us to study the effect of splenectomy in mice which had already developed the full blown Friend Virus Disease syndrome. Mice were inoculated with a 10^{-1} dilution of virus and were divided into 3 groups. The first group was splenectomized 19 days post-inoculation, the second group at 21 days and the third at 27 days. Portions of each group were kept as non-splenectomized controls. Prior to splenectomy, hematocrits, leukocyte and erythrocyte counts were done. Since the same results were seen in the 19 and 27 day groups, Fig. 5 shows data only on mice which were splenectomized 21 days post-infection. There was a striking drop of the elevated hematocrit, erythrocyte and leukocyte levels. The infected non-splenectomized animals maintained the usual increased levels of these parameters.

Discussion. These results indicate that the phase of Friend Virus Disease which is manifested by leukocytosis, increased hematocrit, erythroblastosis and erythrocytosis (hematopoietic response) is intimately related to the spleen. Not only did pre-infection splenectomy prevent this response, but splenectomy of animals with early or already fully established disease, abolished it. In infected splenectomized mice the only suggestion of the hematopoietic response was an increase in erythroblasts in the bone marrow. However, there was no peripheral erythroblastosis and no elevation of hematocrit or erythrocyte levels. Also it was previously demonstrated that no significant increase of Fe^{59} uptake occurred in the bone marrow(1). At no time did the bone marrow in any infected group (control or splenectomized) show evidence of increased activity of the myeloid elements.

The mechanism of splenic control of the hematopoietic response in the Friend Disease is not clear. However, these and previous studies(1) indicate that the hematopoietic

FIG. 3. Count of circulating red blood cells in splenectomized Swiss mice with Friend virus. Legend same as for Fig. 2.

FIG. 4. Percent erythroblasts in bone marrow of Friend virus infected Swiss mice. -●—●-, infected non-splenectomized mice; ■, splenectomized controls without virus inoculation; ▲, mice splenectomized 3 days before virus inoculation; ○, mice splenectomized 4 days after virus inoculation.

FIG. 5. Effect of splenectomy on circulating blood elements in Friend virus infected Swiss mice; virus was inoculated 21 days prior to splenectomy. ●, RBC count; -×--×-, hematocrit; ○, total WBC count.

elements in the spleen are primarily responsible for production of the increased numbers of immature and mature erythrocytes and leukocytes. This is certainly true for the erythropoietic picture and for the leukocytic picture up to 48 days after post-infection. Since at this time the liver and particularly the bone marrow show no significant increase in activity in either splenectomized or non-splenectomized infected mice, the stimulus to hematopoietic activity seems to be specific for those elements in the spleen. One hypothesis is that the virus infects the reticulum (or other target) cells of the spleen and then, by some mechanism, stimulates secondarily the hematopoietic elements of that organ. It is puzzling that the same phenomenon would not occur to the same degree in the bone marrow and liver. However, it may be that the target cells in the spleen are in some way different from morphologically similar cells in the bone marrow and liver, thus accounting for the splenic specificity. If the increased hematopoietic activity were due to a direct action of the virus on the blood forming elements *per se*, one might expect that the bone marrow elements would be similarly stimulated unless again, there were differences among the splenic, liver and bone marrow hematopoietic elements. We feel that the first possibility is a more likely explanation but additional studies are needed before any conclusions are warranted.

Although we believe that the spleen is primarily responsible for the erythropoietic and leukocytic response herein reported, there is some evidence to believe that the liver plays a prominent role in the leukocytosis of splenectomized mice after 48 days post-infection. Note in Fig. 5 that the hematocrit, erythrocyte and leukocyte levels declined progressively from high levels after splenectomy, but at 25 days post-splenectomy or 46 days after inoculation of the virus the leukocytes were reduced but again elevated to 22,000 mm^3. At 55 days post-splenectomy or 76 days after inoculation of the virus (not shown on Fig. 5) the hematocrit dropped to 22%, red blood count to 3.1 million and the white blood count increased to 282,000 mm^3. This increase in white blood count paralleled the development of hepatomegaly. Histological examination of the livers in this group demonstrated at this time uncontrolled proliferation of reticulum cells, few erythrocytic foci and many foci of mature cells of the granulocytic series. There was no evidence of bone marrow hyperplasia and in many instances the bone marrow appeared depressed. It was apparent in this group of mice that splenectomy does prevent the erythropoietic response in the disease but does not prevent eventual development of the malignant reticulum cell leukemia and only delays the appearance of leukocytosis until hepatomegaly occurs.

It is important to stress here that although splenectomy modified the hemapoietic response in the Friend Virus Disease, it did not alter the eventual development of reticulum cell leukemia. Gross(4) has shown, however, that thymectomy either before or after inoculation of "Passage A" leukemic filtrates inhibited viral induction of lymphocytic leukemia in C_3H mice.

Summary. The Friend Virus syndrome in Ha/ICR Swiss is fundamentally modified by splenectomy either 3 days before or 4, 19, 21 and 27 days after inoculation of the virus. Peripheral erythroblastosis and erythrocytosis only occur in presence of the spleen. The characteristic leukocytosis is not seen up to 48 days after infection in splenectomized mice; however, leukocytosis appears again after gross hepatomegaly. Although the hemopoietic syndrome of the Friend Virus Disease is modified by splenectomy, the virus induced reticulum cell leukemia eventually appears.

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