Incidence of Friend Virus-Induced Polycythemia in Splenectomized Mice[1] (37062)

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The inoculation of susceptible mice with the Mirand strain of Friend virus (FV^P) leads to an early erythropoietin-independent polycythemia (1, 2). This response has been described as being greatly modified by splenectomy (3), polycythemia not occurring during the time studied. Splenectomy also affects the response in mice infected with anemia-inducing strains of Friend virus (4, 5). The spleen dependency in FV^P infection has been assumed to be exclusive: splenectomized mice bypass the early erythroid hyperplasia. Since the effect of splenectomy on erythropoiesis in rodents is not dramatic (6), it seemed paradoxical that the role of the spleen should be so exclusive.

During the course of studies on this phenomenon, we have observed that some splenectomized mice do, indeed, become polycythemic subsequent to infection with FV^P, placing the function of the spleen into a quantitative, rather than qualitative role. This report documents these observations.

Methods and Materials. Polycythemia-inducing Friend virus. The virus stock used in these studies is the Mirand polycythemia-inducing strain of the Friend virus complex, passaged in Ha/ICR (Swiss) random bred mice, maintained under liquid nitrogen and used at a titer of 5×10^4 focus-forming units (FFU)/ml (7, 8). Virus was diluted in PBS and injected *via* a lateral tail vein.

Animals. Female inbred DBA/2 and random bred Ha/ICR (Swiss) mice, 5–8 wk old, obtained from the West Seneca animal breeding facility of Roswell Park Memorial Institute, were used throughout these studies. The virus stock was equally infectious in these animals.

Splenectomy. Spleens were surgically removed from the mice under ether anesthesia. At least 2 wk elapsed after splenectomy before other experimental procedures were begun.

Determination of erythropoiesis. Packed red cell volume was determined using tail vein blood collected with heparinized microhematocrit tubes. Hematoxylin and eosin stained tissue sections were prepared after buffered formalin fixation using standard histological techniques. Ferrokinetic studies were done using 0.5 μCi of ^{59}Fe (as ferrous citrate, 25.5 μCi/ml, 0.67 μg Fe/ml, Abbott Laboratories, Chicago, IL) injected *via* a lateral tail vein in 0.5 ml saline. After 24 hr the animals were bled retroperitoneally from the dorsal aorta and an aliquot was diluted to 2 ml with distilled water for ^{59}Fe determination. Selected tissues were removed, cleaned and weighed after sacrifice. In cases of severe hepatomegaly, a weighed portion of the liver was used for the determination of radioactivity. All samples were counted for radioactivity in a well-type crystal scintillation counter (Nuclear-Chicago). Radioactivity in an aliquot of the original ^{59}Fe solution used for injection was similarly determined and uptakes were calculated as the percentage of the initial radioactivity retained, as described previously (9).

Plasma erythropoietin activity. Ha/ICR (Swiss) mice made polycythemic (HCT 58%) by exposure to hypobaric hypoxia for 11 days (0.4 atm for 18 hr/day) were injected intraperitoneally with 0.5 ml pooled, heat inactivated, plasma on two successive days beginning the fourth day after the termination of the hypoxia. The day after the last plasma injection, 0.5 μCi ^{59}Fe was given *via*

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TABLE I. ^{59}Fe Uptake (24 hr) in Exhypoxic-polycythemic Intact and Splenectomized Ha/ICR (Swiss) Given Erythropoietin (ESF).

	No. of animals	% HCT[a]	% Uptake	
			Blood	Femur
Intact	4	66/63	1.1 ± 0.4	0.37 ± 0.04
Splenectomized[b]	5	63/61	1.0 ± 0.2	0.35 ± 0.04
Intact + ESF[c]	9	68/63	13.1 ± 1.5	1.0 ± 0.1
Splenectomized + ESF[ab]	4	65/61	14.9 ± 1.3	1.4 ± 0.3

[a] Hematocrit d.0/d.4. (d.0 = 3 days after termination of hypoxia).
[b] Splenectomy done 2 wk prior to hypoxia.
[c] ESF, 0.5 ml of 1.0 unit/ml, given ip d.0 — 1.

tail vein. Twenty-four hours later blood taken from the abdominal aorta (as above) was determined for radioactivity and uptakes were expressed as a percentage of the injected dose of radioiron.

Results. Since this was a study involving the erythropoietic effects of a polycythemia-inducing virus, we considered it necessary to study, for the sake of valid comparison, similar parameters in intact and splenectomized mice not infected with FV^P but made polycythemic by exposure to hypobaric hypoxia. Erythropoietic responsiveness to 1 unit of sheep erythropoietin (ESF, Step I, Connaught Labs., Ltd., Willowdale, Ontario, Canada) in intact and splenectomized exhypoxic-polycythemic Ha/ICR mice does not differ as determined by 24 hr ^{59}Fe uptake studies (Table I). Splenectomy does not result in a drastic impairment of total erythropoietic capacity.

TABLE II. Occurrence of Polycythemia in Splenectomized[a] Mice Following Infection with Friend Virus (FV^P).

	No. infected	Polycythemic[b]	
		No.	%
Ha/ICR	62	11	18
DBA/2	103	17	16

[a] Splenectomy done at least 2 wk before infection.
[b] Polycythemia defined as hematocrit >58%.

In preliminary studies on the contribution of the spleen to the polycythemic response to infection with FV^P, animals splenectomized and bearing subcutaneous implants of two isogenic spleens became polycythemic following FV^P inoculation later than controls. We therefore considered the possibility that a polycythemic response in totally spleen-deficient animals is delayed, and was not noted previously because of limited observation time. In subsequent experiments, polycythemia (HCT > 58%) occurred in some Ha/ICR and DBA/2 mice infected with FV^P 2 wk after splenectomy (Table II). The data in Fig. 1 show the normal increase in hematocrit following FV^P infection in intact Ha/ICR mice. Similar data were obtained with DBA/2 mice. Shown also are the hematocrit values for the splenectomized DBA/2 and Ha/ICR mice that became polycythemic. They first developed anemia and after a considerable lag, exhibited an increase in their hematocrit. The resulting polycythemia was not as intense and was delayed a minimum of 2 wk. No evidence of residual splenic tissue was found at autopsy in these animals.

The plasma of the splenectomized, FV^P-infected animals contains significant amounts of erythrostimulatory activity during the postinfection period of anemia (Table IV). In contrast, the plasmas of splenectomized, FV^P-infected animals that are not anemic,

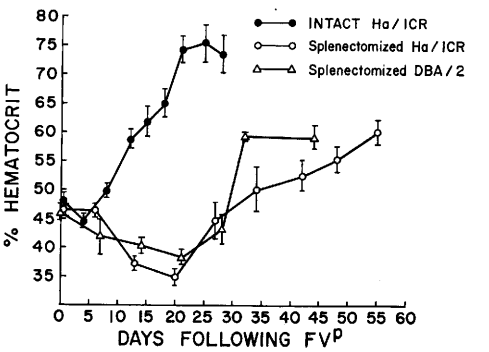

FIG. 1. Percentage of hematocrit in Friend virus (FV^P)-infected intact Ha/ICR mice and splenectomized Ha/ICR and DBA/2 mice that became polycythemic.

TABLE III. Radioiron Uptake (24 hr) in Friend Virus (FV^P)-Infected, Polycythemic Intact and Splenectomized Ha/ICR Mice.

	No. of animals	Av HCT	Liver wt (g)	% Uptake[c] Peripheral blood	Liver	Femur
Intact-$FV^{P[a]}$	7	70	2.5	24.3 ± 1.8	9.8 ± 1.1	0.12 + 0.01
Splenex-$FV^{P[a,b]}$	7	62	5.1	14.6 ± 2.1	28.7 ± 8.0	0.30 + 0.11

[a] 5×10^4 FFU (7,8).
[b] Splenectomy done at least 2 wk prior to virus.
[c] 0.5 μCi ^{59}Fe given via lateral tail vein.

and intact, FV^P-infected animals that are polycythemic do not exhibit this ESF activity.

Radioiron uptake studies show that the liver has a high capacity for total iron incorporation, implicating it as a site for extramedullary erythropoiesis (Table III). Sections of hepatic tissues show many areas of active erythropoiesis (Fig. 2). The liver seems at least in part to be contributing to the increased red cell mass. The low peripheral blood uptake, however, indicates that the spleen deficient animals are less able to produce red blood cells. It may be that the polycythemic response observed may also be partially attributable to an increased half-life for erythrocytes in the absence of the spleen, though available data do not support this (5).

Discussion. The occurrence of polycythemia in splenectomized mice following infection with FV^P makes the role of the spleen in this response less exclusive, and the polycythemia more a function of the presence of an erythropoietic tissue capable of enlargement. As an active erythropoietic tissue, unencumbered as is the bone marrow by a mineralized sheath, the spleen is especially suited to the expansion induced by the erythroid hyperplasia effect of FV^P. However, if the spleen contribution is exclusively a consequence of its unrestricted capacity to increase in size, then it is not surprising that some splenectomized-infected mice become polycythemic. The liver is known to have unexpressed capacity for erythropoiesis, being functional during early development and during stress. The elevation in ESF that occurs during the postinfection anemia in the splenectomized animals may be sufficient to stimulate hepatic erythropoiesis in some animals. This erythropoiesis would then be enhanced by the erythropoietic effects of the virus. Thus, though the erythroleukemia-inducing effects of the virus are ESF-independent (1), the stimulation of extramedullary erythropoiesis by the ESF would provide additional target sites for the virus, leading to the polycythemic response even in the absence of the spleen.

Summary. Polycythemia (HCT 58%) has

TABLE IV. ESF Activity in the Plasma of Splenectomized Ha/ICR (Swiss) Mice Infected with FV^P.[a,b]

Plasma source	Av HCT in donor mice	% 24 hr ^{59}Fe uptake in peripheral blood of assay mice	Av HCT in assay mice[c]
Intact, FV^P-infected	70	1.8 ± 0.3	58
Splenex, FV^P-infected (normal HCT)	43	1.8 ± 0.2	58
Splenex, FV^P-infected (anemic)	24	17.8 ± 1.8	57
Saline	—	1.0 ± 0.4	60

[a] Splenectomy done 2 wk prior to infection with 800 FFU of FV^P.
[b] Plasmas pooled within groups and inactivated at 56° for 30 min.
[c] Ha/ICR, exhypoxic-polycythemic mice used for assay, hematocrit determined at time of sacrifice.

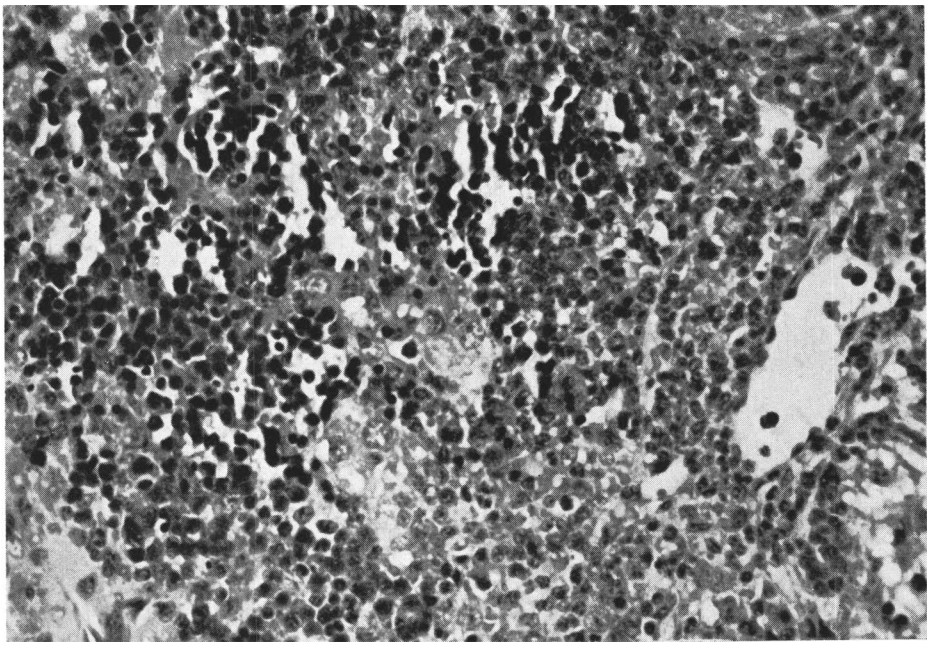

FIG. 2. Hepatic erythropoiesis in Friend virus (FV^P)-infected, splenectomized polycythemic Ha/ICR mouse (H and E stain).

been observed in splenectomized Ha/ICR (18%) and DBA/2 (16%) mice infected with a polycythemia-inducing strain (Mirand) of Friend virus. Data presented indicate that hepatic erythropoiesis, perhaps stimulated by the observed elevations in plasma ESF during the postinfection anemia, contributes significantly to the polycythemia. The polycythemic response appears more contingent on the presence of an expandable erythropoietic tissue than on the presence of the spleen *per se*.

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1. Mirand, E. A., Science **156**, 832 (1967).

2. Mirand, E. A., Steeves, R. A., Lange, R. D., and Grace, J. T., Jr., Proc. Soc. Exp. Biol. Med. **128**, 844 (1968).

3. Mirand, E. A., Hoffman, J. J., Grace, J. T., Jr., and Trudel, P. J., Proc. Soc. Exp. Biol. Med. **107**, 824 (1961).

4. Metcalf, D., Furth, J., and Buffett, R., Cancer Res. **19**, 52 (1959).

5. Brodsky, I., Dennis, L. H., and Kahn, S. B., Cancer Res. **26**, 1887 (1966).

6. Fruhman, G. J., *in* "Regulation of Hematopoiesis" (A. S. Gordon, ed.), Vol. 1, Chap. 16. Appleton-Century-Crofts, New York (1970).

7. Axelrad, A. A., and Steeves, R. A., Virology **24**, 513 (1964).

8. Mirand, E. A., Steeves, R. A., Avila, L., and Grace, J. T., Jr., Proc. Soc. Exp. Biol. Med. **127**, 900 (1968).

9. Mirand, E. A., and Prentice, T. C., Proc. Soc. Exp. Biol. Med **95**, 164 (1957).

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