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Inhibition of Friend Virus (FVP)-Induced Erythropoiesis by an Erythropoiesis-Inhibitory Factor (EIF)[1] (38863)

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Erythropoiesis in Friend virus (Mirand strain, polycythemia-inducing, FV^P) is independent of erythropoietin (ESF) control. As previously described, treatment of mice with antibody to ESF (1) and hypertransfusion-induced plethora (2) are ineffective in eliminating or reducing the response of susceptible mice to FV^P. Furthermore, mice infected with FV^P develop an insensitivity to the erythrostimulatory effects of exogenous ESF (3). That an artificially induced polycythemic state does not result in a reduction of erythropoiesis in FV^P-infected mice does not, however, preclude the possibility that the virus-induced erythrostimulation may be responsive to other erythroregulatory factors. One such factor, an erythropoiesis inhibitory factor (EIF) in a lipoglycoprotein fraction of relatively low molecular weights has been isolated from normal human urine and from urine of a patient with paroxysmal nocturnal hemoglobinuria (4). It has a demonstrated capacity to depress normal erythropoiesis as measured in AKR mice, and has been proposed to act through an ESF pathway (4, 5). It is of interest to determine if this factor is capable of retarding FV^P-induced erythropoiesis. This report documents our findings regarding this question.

Methods and Materials. Animals. AKR/J mice, 4–6 wk old were obtained from the Animal Production Unit of Roswell Park Memorial Institute and used in all studies.

Friend virus. Experimental animals were infected with the Mirand strain (polycythemia-inducing) of Friend virus (6) obtained as a cell-free filtrate from spleens of FV^P-induced leukemic DBA/2 mice. It was used in titers expressed in focus-forming units/ml (FFU/ml) after dilution with phosphate-buffered saline and injected via lateral tail vein.

Erythropoiesis. Red cell production in experimental animals was measured using 0.5 μCi ^{59}Fe (as ferrous citrate, 20.8 μCi/μg Fe, Abbott Labs, North Chicago, IL) injected via lateral tail vein. Twenty-four hours after the administration of radioiron, mice were bled from the retroabdominal aorta under ether anesthesia. After sacrifice, spleens were removed and weighed. Radioactivity in the spleen and an aliquot of peripheral blood was determined using a crystal gamma scintillation counter (Nuclear Chicago). Uptakes of radioiron are expressed as a percentage of the injected dose of radioactivity.

EIF. Methods for the isolation of EIF have been reported previously (4, 5). To test the effect of the inhibitor on FV^P-induced erythropoiesis, 5 mg EIF were given intraperitoneally in 0.2 ml saline on the 14th and 15th days after infection with virus. On the 16th day after virus, each mouse received 0.5 μCi ^{59}FE. Evaluation of iron uptakes proceeded as described above.

Results. Inoculation of AKR/J mice with FV^P resulted in erythrostimulation as evidenced by increased percentage of ^{59}Fe uptake in the peripheral blood by 20 days after infection (Table I). The progressive splenomegaly was similarly reflective of disease induced by FV^P. Although the specific capacity for iron uptake in the spleens of leukemic animals was not elevated significantly (uptake/100 mg spleen wt), total splenic iron uptake increased appreciably. In this series at 20 days the hematocrit of the

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TABLE I. ERYTHROSTIMULATION AND SPLENOMEGALY IN AKR/J MICE INFECTED WITH FV^P.

Days after FV^P[a]	No.	% 24 Hr peripheral blood ^{59}Fe uptake	Spleen wt (g)	% 24 Hr spleen ^{59}Fe uptake/100 mg	Hct (%)
6	5	14.6 ± 3.3	.100 ± .01	3.69 ± .46	38.4 ± 4.4
10	9	10.9 ± 1.4	.294 ± .10	4.44 ± .68	40.3 ± 2.3
15	7	10.5 ± 2.6	.465 ± .14	3.02 ± .42	39.4 ± 2.6
20	9	22.3 ± 2.8[b]	.470 ± .13[c]	3.48 ± .40	41.4 ± 4.1
No FV^P[d]	21	10.3 ± 1.4	.093 ± .01	2.62 ± .33	43.6 ± 1.3

[a] 3500 FFU/0.5 ml iv.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] Saline-injected mice, assayed in groups with experimental, FV^P-infected mice.

TABLE II. EFFECT OF EIF ON FV^P-INDUCED ERYTHROPOIESIS IN AKR/J MICE.[a]

Group	No.	% 24 Hr peripheral blood ^{59}Fe uptake	Spleen wt (g)	% 24 Hr spleen ^{59}Fe uptake/100 mg	Hct (%)
FV^P + Saline	8	28.9 ± 1.7[b]	0.847[c]	2.58 ± .34	59
FV^P + EIF	5	16.6 ± 3.1	0.235[c]	6.78 ± .54[d]	53
No FV^P[e]	7	15.3 ± 1.4	0.060[c]	1.66 ± .14	48

[a] Mice infected with 3000 FFU iv. Two 5-mg injections of EIF in 0.2 ml saline given the 14th and 15th days after FV^P-infection. ^{59}Fe given the 16th day (0.5 μCi iv) and uptakes determined 24 hr later.
[b] Significantly greater than both saline control ($P < 0.001$) and EIF-treated group ($0.001 < P < 0.005$).
[c] All spleen values significantly different from each other ($P < 0.001$).
[d] Significantly greater than both FV^P-saline and No-FV^P groups ($P < 0.001$).
[e] Saline-injected, normal AKR/J mice, not receiving EIF.

infected mice was not affected, no difference being significant.

In normal AKR mice the addition of EIF, 5 mg/day for 2 days resulted in a significant decline in the uptake of ^{59}Fe as measured in the peripheral blood (5). To test the effect of the inhibitor in FV^P-infected AKR mice, 5 mg EIF were injected intraperitoneally on the 14th and 15th days after virus. Radioiron was given on the 16th day and uptakes determined 24 hr later. The 14th and 15th days were chosen for EIF treatment in the light of the data on the AKR response to FV^P indicating that erythrostimulation is evident between the 15th and 20th days after inoculation with virus and knowing the reaction rates for EIF (5). With injections of EIF given the 14th and 15th days, uptakes were determined 17 days after virus. As evidenced by the data in Table II, the presence of EIF led to a reduction in peripheral blood radioactivity and splenomegaly. Peripheral blood percentage of ^{59}Fe uptake in FV^P-infected mice receiving EIF did not differ from that in mice not receiving any virus. Both groups were significantly lower than the group receiving virus only. Spleen weight was reduced in the group receiving EIF, but still increased, compared with the group not receiving virus. There was a substantial increase in the specific percentage of radioactivity in the spleens of the virus-infected animals receiving EIF. However, total spleen uptake was lower than that for the virus-only group (total uptake = spleen weight × uptake/100 mg spleen weight). It should be noted that the series of experiments in Table II were different from those reported in Table I. For this reason, uptakes are not necessarily directly comparable from one assay to another, one group of animals to another, one virus stock to another (note difference in control uptakes).

Discussion. Previous experiments have shown that a human urine-derived lipoglycoprotein (EIF) was capable of inhibiting erythropoiesis in AKR mice when administered directly to experimental animals.

Additional studies indicated that 0.25 mg of the EIF used in the experiments described herein could neutralize the stimulating effect of 0.4 ± 0.01 IU of ESF when preincubated together prior to injection. These later data could lead to the interpretation that the EIF exerted its inhibitory effect by blocking erythrostimulation by ESF. To test this possible mechanism of inhibition, erythropoietin-independent erythropoiesis is necessary. Such is the case with FV^P-induced erythroleukemia, and the data reported herein suggest that the inhibitory effect of EIF is independent of ESF. The elevation in specific uptake of ^{59}Fe in the spleen could indicate that the EIF has led to erythroid maturational arrest or lack of release. However, both these are difficult to reconcile with the decrease in splenic weight. The decreased hematocrit would support either, and it is possible that the increased percentage of ^{59}Fe uptake/100 mg spleen weight is only a transitory phenomenon consequent on initial treatment with EIF.

The inhibitory effect of EIF in experimental malignant erythropoiesis is of potential significance. Reduction of both peripheral blood radioactivity and splenomegaly in the leukemic mice in response to an erythroinhibitory factor may indicate that the EIF could be of therapeutic value. More extensive studies on the long-term effect of EIF, when given intermittently to FV^P-infected mice, would seem indicated. It has already been shown that normal AKR mice can tolerate prolonged exposure to the EIF without adverse effect. Although experiments are still to be done defining the specificity and mechanism of action of EIF the potential for the use of physiological regulators for the control of erythropoietic dyscrasias, especially polycythemia vera for which FV^P is an excellent model, is encouraging.

Summary. An erythropoietin-independent murine erythroleukemia (FV^P) has been used to evaluate the effects of an erythropoiesis-inhibitory factor (EIF) isolated from human urine. The consequent inhibition of FV^P-induced erythropoiesis suggests that EIF exerts its effect independently of ESF. The inhibitory effect of EIF on FV^P-induced erythropoiesis may reveal a potential for the physiological control of some types of erythroleukemia.

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