serum protein higher in M pigs throughout.

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Spleen Focus Formation by Polycythemic Strains of Friend Leukemia Virus* (32831)

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During the past 6 years a number of passage lines of Friend virus (1) have been developed and carried in this laboratory which may be distinguished by their effects on erythropoiesis. In susceptible strains of mice all of the lines induce marked hepatosplenomegaly, increased numbers of large mononuclear cells in the blood and spleen, and elevated ^{59}Fe uptakes in blood, spleen, and liver. However, some lines (FV-P) induce a hypervolemic polycythemia within 2–3 weeks of infection (2,3), while others (FV-A) cause anemia after 4–5 weeks. Because lines of the latter type in our laboratory had been obtained after passage of the former type through rats (4) or after recovery of virus from the progeny of infected pregnant mice (5), the pos-

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sibility was considered that more than one virus could have been present in the virus preparations received from Dr. Charlotte Friend.

For an approach to the study of this possibility, the spleen focus assay method (6) was chosen because of the rapid, enumerative response observed in susceptible hosts. In addition, the direct proportionality between the number of foci per spleen and the dose of virus administered implies that focus formation may be initiated by only one infectious entity in Friend virus preparations. It was, therefore, of interest to determine whether the 2 types of Friend virus described above differed as well in their abilities to initiate spleen focus formation.

Materials and Methods. Virus. Friend virus was originally obtained through the courtesy of Dr. Charlotte Friend, and has been carried by serial passage in Ha/ICR Swiss mice in our laboratory. Virus preparations routinely used for infection of newborn or adult mice were cell-free filtrates obtained from a 10% suspension of homogenized spleen from mice with Friend disease. The spleens were homogenized in cold Locke–Ringer (LR) solution, the homogenate was clarified at 5000*g*/10 min at 4°C and the supernatant fluid was passed through a HA-grade millipore filter (average pore size 0.45 μ).

The spleen focus assay method for titrating Friend virus (6) was carried out by injecting serial dilutions of virus (in LR solution) into the tail veins of adult mice (0.5 ml). Nine days later the mice were sacrificed, their spleens removed and fixed overnight in Bouin's fluid. Discrete foci on the surfaces of the intact spleens were then counted with the unaided eye. Virus titers are expressed in focus-forming units (FFU)/ml (where 1 FFU is that amount of virus required to induce an average of 1 focus/spleen).

Mice. The Ha/ICR Swiss mice of both sexes between 4 and 8 weeks of age were obtained from our own breeding colonies. Each experiment was carried out with mice of the same sex and within an age range of 1 week. Some mice were plethorized before infection by 3 daily i.p. injections of 0.5 ml of washed, packed, homologous red blood cells, and elevated hematocrits were maintained after infection by weekly injections of the same material.

^{59}Fe uptake studies. Two or 3 weeks after virus infection, 0.5 μC of ^{59}Fe in 0.2 ml unbuffered 0.9% NaCl solution was injected into the jugular vein of each mouse (7). Twenty-four hours later the mice were bled by aorta and their spleens removed and weighed. Radioactivity in blood and spleen, as well as in an aliquot of the original ^{59}Fe used for injections, was measured in a Baird Atomic scintillation counter. Uptakes of ^{59}Fe in the blood and spleens were calculated as described previously (8).

Results. The strain of Friend virus (FV-P) which induces stem cell leukemia and polycythemia in Swiss mice may induce hematocrits of over 80% by 3 weeks postinfection (3). Occasionally, however, some of these infected mice become anemic and passage of virus from these animals usually provokes a wide range of hematocrit values. Sometimes, a passage line (FV-A) may be developed which consistently induces anemias. Several stable Friend virus lines have been developed and tested: 5 which induce anemia, 1 which induces no consistent hematocrit change, and 3 which induce polycythemia (Table I). Splenic cell-free filtrates from these stable lines were prepared in the usual fashion, diluted as indicated, and 0.5 ml volumes injected i.v. into groups of mice. Nine days later the recipient spleens were scored and the titers of each filtrate were calculated in FFU/ml. Virus with focus-forming activity was found in high titer in all polycythemic lines, in low titer in the line which induced no consistent hematocrit change, and in 2 of the anemic lines. No focus-forming activity was detected in the remaining 3 anemic lines which, after 3 blind cell-free passages at 9 day intervals, still failed to show focus-forming activity.

To study in greater detail the association between spleen focus formation and elevated hematocrits, we injected mice with various mixtures of the FV-P and FV-A lines. Some of these mice were sacrificed 9 days later and scored for the number of foci in their spleens, while the others were kept for 4 weeks before their hematocrit levels were determined. In Table II, the results show that the number of

TABLE I. Focus-Forming Ability of Anemic and Polycythemic Strains of Friend Leukemia Virus.

Virus line (laboratory)	Effect on host hematocrits[a]	Dilution[b]	No. foci/spleen	Virus titer[b] (FFU/ml)
FV-A (Mirand)	anemia	10^{0}	0,0,0,0,0	0
FV-A (BB13-Mirand)	anemia	10^{0}	0,0,0,0,0	0
FV-A (BCL-Mirand)	anemia	10^{-1}	0,0,0,0,0,0	0
FV-A (Friend)[c]	anemia	10^{-2}	0,0,0,4,8,10	740
FV-A (Rich)[c]	mild anemia	10^{-3}	0,0,0,0,1,3	1,340
FV (Fieldsteel)[c]	no change	10^{-2}	0,2,3,15	1,000
FV-P (Axelrad)[c]	polycythemia	2×10^{-4}	0,0,0,1,3,5,9	26,000
FV-P (S-Mirand)	polycythemia	3×10^{-4}	0,2,11,12,15	53,000
FV-P (Mirand)	polycythemia	10^{-4}	0,2,3,4,4,4,7,8	80,000

[a] All hematocrits were carried out 30 days postinfection.

[b] Virus titers and dilutions were calculated with reference to cell-free filtrates of 10% suspensions of homogenized leukemic spleens as described in "Materials and Methods." Dose injected was 0.5 ml iv.

[c] Kindly supplied by: (respectively) Dr. C. Friend, Mt. Sinai Hospital, New York; Dr. M. Rich, Albert Einstein Medical Center, Philadelphia; Dr. F. Price of this Institute; and Dr. A. Axelrad, Ontario Cancer Institute, Toronto.

TABLE II. Correlation among FV-P Concentration, Number of Foci per Spleen, and Blood Hematocrits in Ha/ICR Mice.

Virus mixtures injected		No. of foci per spleen/ blood hematocrits[b]	Mean no. of foci per spleen/ mean hematocrit value
FV-P[a]	FV-A[a]		
0	1.00	0,0,0,0,0,0,0,0 46,46,47,47,47,48,49,49	0.0 47
0.25	0.75	0,0,0,0,0,0,1,2,2,8 48,49,51,51,52,57,63,65,79	1.3 57
0.50	0.50	0,0,0,0,0,4,6,7,7,14 42,49,51,51,56,70,70,78,79	3.8 61
0.75	0.25	0,0,5,8,11,11,15,18,24,32 48,50,54,56,67,73,75,78,78,82	12.4 66
1.00	0	0,0,0,6,14,14,21,25,28,31 46,47,48,49,65,69,72,78,84	13.9 62

[a] FV-P and FV-A lines were both diluted 2×10^{-3} from filtrates of 10% spleen homogenates prior to mixing; — mixtures are expressed as fractions of the total dose.

[b] Half of the mice were sacrificed 9 days postinfection and their spleens counted; blood hematocrits were measured from the remainder 28 days postinfection.

spleens bearing foci at 9 days was usually about the same as the number of high hematocrits for the same dilution mixture. In addition, virus mixtures which induced high focus counts also induced high hematocrits.

Recently, it has been shown that the polycythemic virus (FV-P) differs from anemic lines (FV-A) in another respect: Following infection of plethorized mice, FV-P initiates the resumption of erythropoiesis, as judged by increased ^{59}Fe uptake in the spleen and blood, while FV-A and Rauscher virus do not (2). The relationship between this criterion of FV-P infection and spleen focus formation

TABLE III. Comparison of ^{59}Fe Uptakes in Plethorized Mice and Spleen Focus Formation Induced by Different Virus Strains.

Virus[a]	Effect on host hematocrits	Mean no. of foci per spleen (9 days postinfection ± SE)		Mean 24-hour ^{59}Fe uptakes (%) in plethorized mice (14 days postinfection ± SE)	
		Normal mice	Plethorized mice	Spleen	Blood
Control (no virus)	—	0.0	0.0	0.6 ± 0.1	0.7 ± 0.1
FV-A (Mirand)	Anemia	0.0	0.0	0.7 ± 0.1	0.7 ± 0.1
RV	Mild anemia	5.4 ± 1.6	0.4 ± 0.1	4.7 ± 3.7	0.8 ± 0.3
FV-P (Mirand)	Polycythemia	27.0 ± 8.5	4.8 ± 1.5	15.9 ± 9.3	5.8 ± 3.2

[a] Each virus preparation was diluted 10^{-2} from a 10% spleen filtrate, and 0.5 ml injected iv into young adult Ha/ICR mice. RV = Rauscher virus, kindly supplied by Dr. Frank J. Rauscher, National Cancer Institute, Bethesda.

is demonstrated in Table III. Different virus strains, such as FV-P, FV-A and the Rauscher virus (RV), were injected into groups of plethorized and normal mice, and the number of foci per spleen 9 days postinfection were compared with the % ^{59}Fe uptakes in spleen and blood 2 weeks postinfection. A clear association is shown between the presence of spleen foci in normal or plethorized mice and increased ^{59}Fe uptakes in the blood and spleens of plethorized mice.

Discussion. These data demonstrate a close relationship between a virus (FV-P) which is capable of rapidly inducing polycythemia in susceptible strains of mice (2), and a virus which can initiate spleen focus formation (6) in the same strains. It appears that both viruses are the same (Tables I and III), and if this were so, then spleen focus formation would become a more sensitive indicator of infection than either the development of polycythemia or the resumption of erythropoiesis in plethorized mice. However, one cannot exclude the possibility that these viruses are distinct, but tend to express themselves together. In either case, we believe that the most appropriate term for the virus with focus-forming activity is SFFV (for spleen focus-forming virus), and that as a working hypothesis until proved otherwise, SFFV is the same as the formerly described polycythemic virus, FV-P, that was originally obtained from a preparation of Friend virus (2).

It is interesting to note that some anemic strains of Friend and Rauscher viruses showed a low titer of SFFV; however, high titers of SFFV in Friend virus preparations (10^4 FFU/ml or above) have always rapidly induced polycythemia in susceptible mice at our laboratory. The antigenic identity of SFFV obtained from Friend and Rauscher preparations has been shown previously by virus neutralization studies (9); it remains to be determined if any viruses antigenically distinct from SFFV share its capacity to initiate spleen focus formation.

The role of SFFV, in combination with other murine viruses, as an agent responsible for the variety of pathological syndromes ascribed to the action of Friend and Rauscher virus preparations (1, 10–16) has yet to be clarified. Current studies directed towards solving this problem are complicated by the probability that FV-A is present in most, if not all, SFFV preparations, and by the possibility that it may function as a helper virus in the action of SFFV.

Summary. Several strains of Friend virus were tested for their ability to initiate focus formation in the spleens of susceptible mice. Strains which induce polycythemia had high focus-forming activity, while strains which induce anemia had little or no focus-forming activity. A positive correlation was also demonstrated between virus strains with focus-forming activity and those which initiate the resumption of erythropoiesis in plethorized mice. It is concluded that most Friend virus preparations contain at least 2 viruses: one which has spleen focus-forming activity

(SFFV) and which probably is responsible for inducing polycythemia when present in high titers, and another which, when active in the presence of little or no SFFV, induces anemia. The role of either virus in initiating leukemia remains to be determined.

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Effects of Thorotrast upon the Reactivity and Intravascular Disappearance Rate of Fibrinogen in the Rabbit* (32832)

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The experiments reported herein are an extension of earlier studies from this laboratory of the *in vitro* (1) and the *in vivo* (2) effects of Thorotrast upon hemostatic factors in the rabbit. In those studies Thorotrast was found to prolong the thrombin time of plasma but not to lower its fibrinogen level. Further evidence of the effect of Thorotrast upon fibrinogen reactivity is presented below along with measurements of the effect of Thorotrast upon the intravascular disappearance rate of ^{131}I-labeled fibrinogen. One purpose was to determine if the return of the thrombin time to normal after Thorotrast was associated with the removal from the circulation of increased amounts of fibrinogen altered by Thorotrast.

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A second purpose was to look for evidence of intravascular clotting after Thorotrast. Our recent studies indicated that measurement of the disappearance rate of ^{131}I-labeled fibrinogen may detect intravascular clotting after endotoxin in rabbits whose fibrinogen levels do not fall. Both the injection of Thorotrast (2) and of tissue thromboplastin (3) lower Factor V, Factor VIII, and platelet levels in the rabbit. The possibility existed that the falls induced by Thorotrast might stem not only from its direct action upon these factors but also from intravascular clotting.

Thorotrast is used to prepare rabbits for the deposition of fibrin in glomerular capillaries and renal cortical necrosis (the generalized Shwartzman reaction) by agents known to promote intravascular clotting, e.g., endotoxin (4), activated Factor X (5), and by agents under study for their ability to promote intravascular clotting (6). It has been assumed that the intravascular clotting is induced by only the second agent and that