ERYTHROLEUKEMIA INDUCTION BY FRIEND LEUKEMIA VIRUS

A Host Gene Locus Controlling Early Anemia or Polycythemia and the Rate of Proliferation of Late Erythroid Cells*

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Friend leukemia virus (FLV)[1] (1) is a C-type murine retrovirus that induces rapid effects on erythropoiesis in susceptible mice (1–5). Like other acute leukemia viruses, FLV is a complex of two viruses, a replication-defective component(s) Friend spleen focus-forming virus (SFFV) (5) and a replication-competent virus, F-MuLV (4–7). Recent studies (8, 9) using molecularly cloned SFFV DNA have indicated that erythroleukemia can be induced by F-MuLV and a single SFFV genome. The erythroleukemia induced by FLV can be divided into at least two stages: an early stage of rapid splenomegaly at 1–3 wk post-infection (1–5, 9) and a more "malignant" stage of 3 wk or over, during which tumorigenic erythroleukemic cell lines can be detected in the infected spleens (10–15). Other hematological changes can also be recognized during the early phase of the disease. For example, the rapid splenomegaly is accompanied by extensive proliferation and differentiation of immature erythroid cells and the appearance of large numbers of cells capable of forming erythroid colonies (CFU-E) in vitro (16–18, 4).

In addition to these hematological aberrations, a rapid change in the hematocrits of these mice can also be detected early in the disease (1, 2, 4). The direction of these changes, however, can be different in response to two distinct strains of FLV (1, 2, 4). After infection of the original Friend isolate of FLV (1) (FV-A), the mice rapidly developed anemia at 2–3 wk after infection. However, a distinct strain of FLV (FLV-P) was subsequently isolated by Mirand (2) that is capable of inducing a rapid and severe polycythemia instead of the anemia. These contrasting changes in the hematocrits of the infected mice were found to be a function of the defective components $SFFV_A$ and $SFFV_P$, which are associated with FV-A and FV-P complexes, respectively (4, 5). In addition to a difference in hematocrits of the infected mice,

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[1] *Abbreviations used in this paper:* BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; CFU-E*, CFU-E from virus-infected mouse; epo, erythropoietin; FFU, focus-forming unit; FLV, Friend leukemia virus; F-MuLV, replication-competent Friend helper virus; FV-A, anemia-inducing isolate of Friend leukemia virus complex; FV-P, polycythemia-inducing isolate of Friend leukemia virus complex; PFU, plaque-forming unit; RBC, erythrocytes; SFFV, spleen focus-forming virus; $SFFV_A$, replication-defective transforming virus contained in preparation of FV-A; $SFFV_P$, replication-defective transforming virus contained in preparation of FV-P.

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there is also a difference in the response of the erythroid progenitors of these mice to the hormone erythropoietin (epo) (4, 16–18) after infection by FV-A or FV-P. Although erythroid progenitor cells in FV-A-infected mice require epo to proliferate (4, 19, 20), the erythroid progenitors detected in susceptible mice early after infection with FV-P are independent of epo (4, 16–18).

In the present communication, we investigated the host contributions to these early changes in erythropoiesis. This study indicates that in addition to the viral contributions, host genes also play an important role in determining the outcome of the early changes induced by FLV. In a preliminary report (21), we described the identification of a host gene locus, *Fv-5*, that determines the induction of early anemia or polycythemia by FV-P. In this report, we confirmed these findings and extended the study to investigate the possible modes of action of the locus. It was determined that this locus, *Fv-5*, may control the level of erythrocytes by regulating the rates of proliferation of a relatively mature (post CFU-E) erythroid precursor cell in the spleens of the mice.

Materials and Methods

Mice. Female mice of strain CBA and DBA/2, aged 6–10 wk, were obtained from The Jackson Laboratory, Bar Harbor, ME.

Virus. Clonal isolation of FV-P and FV-A was described elsewhere (4). $SFFV_A$ and $SFFV_P$ titers (FFU_S) were determined by spleen focus-forming assay in DBA/2 mice (5). The $F\text{-}MuLV_P$ clone used in this study is an NB-tropic clonal isolate obtained after end-point dilution of FV-P (4, 6). Titers of $F\text{-}MuLV_P$ were determined by XC plaque assay and expressed in plaque-forming units (PFU)/ml (6, 22).

Clones of RAT-1 cells nonproductively infected with $SFFV_A$ (P5) (4) and $SFFV_P$ (501) (4) virus were rescued by infection with $F\text{-}MuLV_P$ at a multiplicity of 0.1 PFU/cell (6). Culture supernatants were harvested after the cells had been passaged by trypsinization and replating two or three times after infection.

Molecularly cloned $SFFV_P$ DNA was obtained as previously described (9). After calcium phosphate-mediated transfection of the $SFFV_P$ DNA fragment into NIH/3T3 cells, $F\text{-}MuLV_P$ was used to rescue the $SFFV_P$ components, and the culture fluid containing rescued virus was harvested and inoculated into DBA/2 or CBA mice (9).

Culture of CFU-E or CFU from Virus-infected Mice (CFU-E).* The assay for colony-forming unit erythroid (CFU-E or CFU-E*) was performed as described previously (23). In short, bone marrow cells were obtained by flushing femurs with α-medium. Spleen cell suspensions were made by pressing spleen fragments with glass slides. After washing with α-medium, the bone marrow or spleen cells were plated in 1 ml of 0.8% methylcellulose in α-medium supplemented with 7.5×10^{-5} M 2-mercaptoethanol, 1% deionized bovine serum albumin (Sigma Chemical Co., St. Louis, MO), and 30% fetal calf serum (Flow Laboratories Inc., McLean, VA). Erythropoietin (lot 3038; Connaught Laboratory, Toronto, Canada) was added at a final concentration of 0.5 U or as specified. Colonies containing 8–64 hemoglobinized cells at day 2 were counted as CFU-E or CFU-E*.

Colony Assay for Friend Virus-induced Tumorigenic Cells (CFU-FV). The assay for CFU-FV was performed as described previously (13). Briefly, cells (1×10^6 cells/ml) from individual spleens were cultured in 1.3% methylcellulose in Iscove's modified Dulbecco's medium (24) with 30% heat-inactivated fetal calf serum and 1×10^{-5} M 2-mercaptoethanol. Dishes were incubated at 37°C for 14 d, at which time macroscopic colonies (containing 10^4–10^5 cells) were scored by eye.

Assay of Hematocrit, Erythrocytes, and Reticulocytes. For hematocrit assays, blood samples were taken from tail veins of the mice every week with heparinized hematocrit tubes (American Dade Divison, American Hospital Supply Corp., Miami, FL). Erythrocytes (RBC) were counted with the aid of a hemocytometer, and reticulocytes in the peripheral blood, bone marrow, and spleen cell suspensions were stained with new methylene blue and counted at weekly intervals.

Assays of Erythroblasts in the Bone Marrow and Spleens. Bone marrow and spleen cells were suspended in phosphate-buffered saline (phosphate, 0.02 M, NaCl 0.14 M; pH 7.2) containing 1% bovine serum albumin at the concentration of 1×10^6 cells/ml, and 0.1 ml of these suspensions were spun onto a slide glass. After air drying, the cells were fixed with methanol and stained with Giemsa staining solution, and the number of erythroblasts was counted.

Uptake of ^{59}Fe by the Bone Marrow and Spleens. Mice were injected with 0.1 μCi of radioiron ($[^{59}Fe]Cl_3$) in 0.5 ml of phosphate-buffered saline. 6 h after injection, the mice were killed. The spleens and femurs were removed, and radioactivity was assayed as described elsewhere (25). Data was expressed as percent of radioiron injected.

Results

Hematocrit Changes in DBA/2 and CBA Mice after Infection with FV-P and FV-A Preparations from Different Sources. In a preliminary communication (21), we have shown that after infection of DBA/2 and CBA mice with FV-P, contrasting changes of hematocrits could be observed in these two strains of mouse. To ensure that these contrasting effects were induced by the $SFFV_P$ genome, FV-P stocks were obtained by a variety of methods. Results in Table I indicate that after infection of FV-P virus, a severe polycythemia developed in DBA/2 mice, whereas a rapid anemia was induced in the latter. This result confirms our preliminary report (21) that contrasting changes of hematocrit are induced in these two strains of mouse. Anemia was induced in both strains of mouse when FV-A virus was used. To ensure that the FV-P used was not contaminated with $SFFV_A$, two rat cell clones, P5 (4), nonproductively infected with $SFFV_A$ and 501 (4), nonproductively infected with $SFFV_P$, were rescued with cloned F-MuLV, and the resulting virus preparations were used. Results indicated again that anemia developed in CBA mice, whereas polycythemia developed in DBA/2 mice when $SFFV_P$ was rescued. $SFFV_A$ rescued with F-MuLV induced

TABLE I

*Hematocrit Values of DBA/2 and CBA Mice 2 wk after Infection with FLV**

	Percent hematocrit	
	DBA/2	CBA
Uninfected	51.0 ± 1.0	49.5 ± 0.5
FV-P‡	66.5 ± 0.9	36.8 ± 3.7
FV-A‡	40.0 ± 2.0	34.5 ± 7.3
F-MuLV‡	47.3 ± 1.5	47.5 ± 3.5
F-MuLV + rat nonproducer 501 ($SFFV_P$)‡	65.3 ± 3.7	41.9 ± 5.3
F-MuLV + rat nonproducer P5 ($SFFV_A$)‡	45.0 ± 1.6	39.3 ± 1.5
F-MuLV + NIH/3T3 cells (Transfected with $SFFV_P$ DNA)§	73.0 ± 0	30.0 ± 5.9
Spleen extracts of CBA Mice (FV-P)‖	69.7 ± 2.5	41.2 ± 4.0

* Virus preparations were injected into 6–10-wk-old DBA/2 or CBA mice through the tail veins. 2 wk after infection, the hematocrit values were determined. At least four mice were used in each determination.

‡ Virus preparations of FV-P (2×10^3 FFU), FV-A (10^3 FFU), F-MuLV (2.5×10^6 XC plaque units), and rat nonproducer cell clones 501 and P5 were described previously, as in ref. 4. Rescue of the nonproducer cell clones is described in Materials and Methods.

§ Transfection of NIH/3T3 cells with molecularly cloned $SFFV_p$ DNA and rescue of the NIH/3T3 cells was described in ref. 9.

‖ 6-wk-old mice were injected with FV-P (2×10^3 FFU) (4). 2 wk later, the mice were killed and a 10% spleen extract was obtained from the spleens of these mice. The extract was filtered through a 0.45-μM millipore filter before use.

anemia in both strains of mice. To further rule out the possibility of any $SFFV_A$ viral genomes in the $SFFV_P$ nonproducer cell clone 501, we transfected molecularly cloned $SFFV_P$ DNA into NIH-313 cells and rescued the transfected cells with cloned F-MuLV (9). As can be seen in Table I, this virus preparation, which contains only $SFFV_P$ and cloned F-MuLV, also induced anemia in CBA mice and polycythemia in DBA/2 mice (Table I). Finally, there is the possibility that after infection of FV-P into CBA mice, certain host-induced changes in the SFF_P viral genomes occurred, or perhaps endogenous $SFFV_a$-like viruses were induced. To examine this possiblity, spleen extracts of anemic CBA mice 2 wk after infection with FV-P were injected into DBA/2 and CBA mice. Results in Table I indicate that, similar to the original FV-P, this spleen extract was capable of inducing a severe polycythemia in DBA/2 mice and a rapid anemia in CBA mice. These results indicate that the induction of these contrasting changes in hematocrits in DBA/2 and CBA mice is a function of the $SFFV_P$ genome.

Levels of Circulating Reticulocytes and RBC in CBA and DBA/2 Mice after Infection with FV-P and FV-A. The kinetics of changes in the levels of mature erythroid cells, reticulocytes, and RBC in the peripheral blood of these mice were examined. DBA/2 and CBA mice were infected with FV-P, and the levels of reticulocytes and RBC were measured at different intervals after infection. Results showing the average numbers of reticulocytes and RBC are illustrated in Fig. 1. The data indicate that after infection with FV-P, there was an increase in the levels of RBC in the blood of DBA/2 mice and a corresponding decrease in the level in the blood of the CBA mice, accounting for the differences in the observed hematocrits in these infected mice. Examination of the levels of reticulocytes in the blood of these infected mice also revealed similar changes, with a rise in the reticulocyte levels in DBA/2 mice and a drop in reticulocytes in the CBA mice during the first 2 wk. Results of experiments after the kinetics of changes in RBC and reticulocytes levels in the individual infected DBA/2 or CBA mice also revealed similar patterns of changes (Fig. 1 b). Subsequent to infection of FV-A, however, the level of RBC decreased in both CBA and DBA/2 mice, accounting for the reduction of hematocrits in these mice (Fig. 2). Changes in the levels of reticulocytes in these FV-A-infected mice was similar to those of FV-P-infected mice, although the number of reticulocytes in FV-A-infected DBA/2 mice did not increase to the same level as those in FV-P-infected animals.

Erythroid Progenitor Colonies (CFU-E) in DBA/2 and CBA Mice Infected and Uninfected with FV-P. It is possible that these differences in erythropoiesis might be the reason for the changes in hematocrit levels in these infected mice. These studies were performed in DBA/2 and BALB/c mice. To examine whether there are differences in erythropoietin requirement of the CFU-E* in DBA/2 and CBA mice, we examined the epo requirements of the CFU-E formation in infected and uninfected CBA and DBA/2 mice. Results of these investigations are illustrated in Figs. 3 and 4. Although there was a slight difference in the number of CFU-E in the absence of any added epo, the epo dose-response curves were essentially the same for the CFU-E in the uninfected DBA/2 and CBA mice. Without any added epo, ~20 CFU-E per 10^5 nucleated cells could be detected in CBA mice, whereas no detectable CFU-E was found in DBA/2 mice in the absence of any added hormone. However, other than this small difference, the epo dose-response curves for the CFU-E in both strains of mice were very similar, reaching a maximum of ~200–250 colonies/10^5 cells at ~0.2

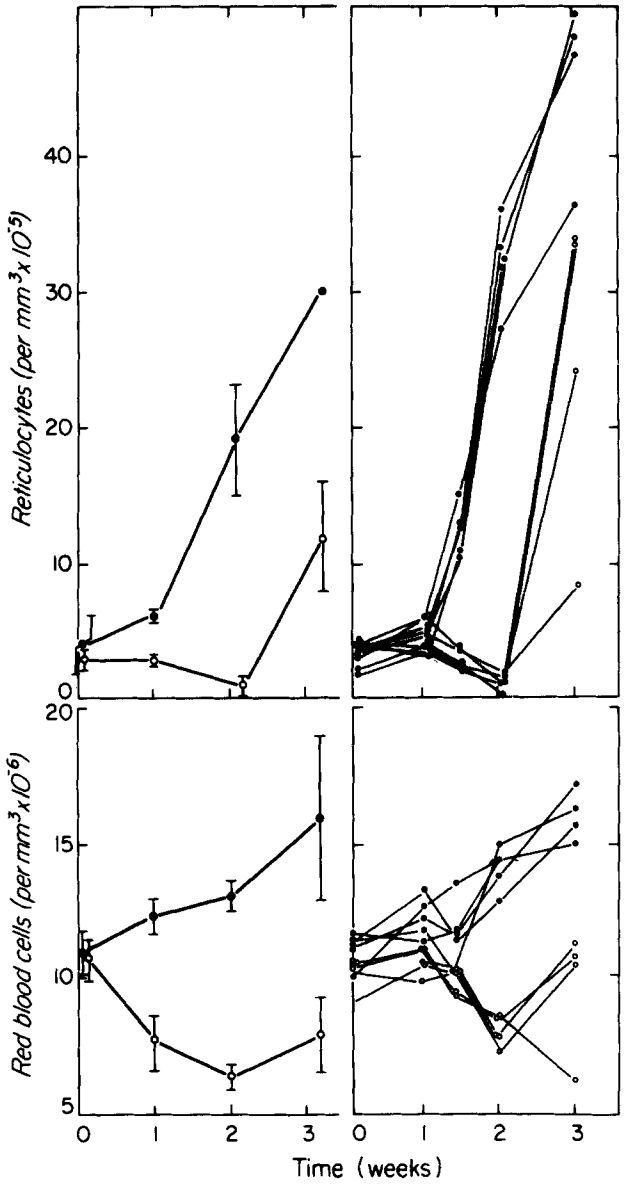

FIG. 1. Kinetics of change in the levels of reticulocytes and RBC in the peripheral blood of CBA and DBA/2 mice infected with FV-P. CBA and DBA/2 mice were infected with 2×10^3 FFU of FV-P. At various times after infection, the levels of reticulocytes or RBC were determined in the peripheral blood of these cells. Data from two separate experiments are illustrated. The results from panel b shows kinetic changes of individual mice. ●, DBA/2 mice; ○, CBA mice.

U/plate. We next examined the epo dependence of CFU-E* formation in the two strains of mouse infected with FV-P. Equal doses of FV-P were injected into CBA or DBA/2 mice, and the levels of CFU-E in the spleens and marrow of these animals were measured in the presence or absence of 0.5 U/plate of epo at 0, 1, 2, or 3 wk after infection. Results are illustrated in Fig. 4. The data in Fig. 4a showed all of the

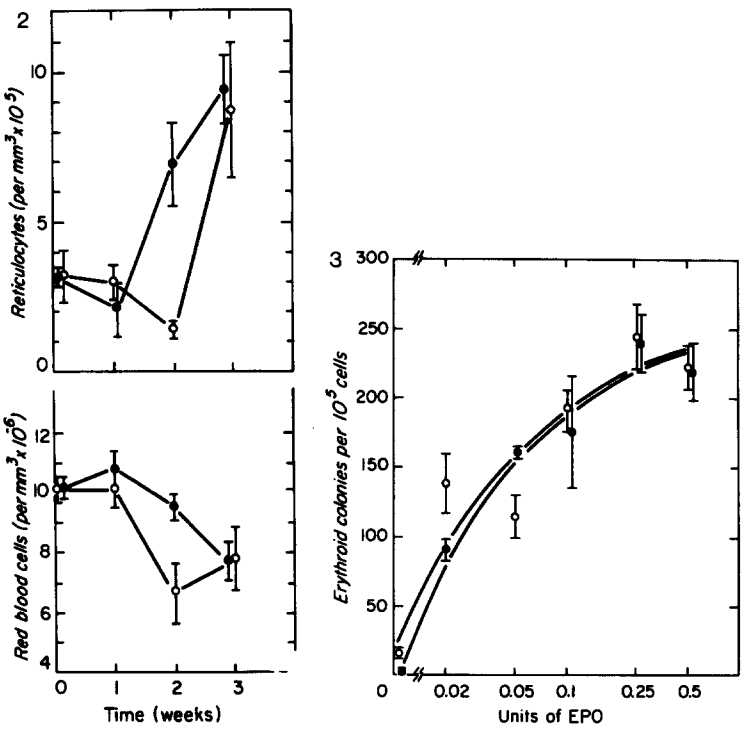

FIG. 2. (left). Kinetics of change in the level of reticulocytes and RBC in the peripheral blood of CBA and DBA/2 mice infected with FV-A. Same as Fig. 1, except 10^3 FFU of FV-A was used. ●, DBA/2 mice; ○, CBA mice.

FIG. 3. (right). Dose-response curves of epo in the formation of CFU-E in marrow cells from CBA and DBA/2 mice. Bone marrow cells were obtained from 6-wk-old CBA or DBA/2 mice and plated for CFU-E in the absence or presence of different concentrations of epo, as described in Materials and Methods. ●, DBA/2 mice; ○, CBA mice.

CFU-E* in the spleens of these animals were under the control of epo at the time of infection. This was then followed by a dramatic increase in the number of epo-independent erythroid colonies as early as 1 wk post-infection in both mouse strains. Results from the bone marrow of these infected animals are summarized in Fig. 2b. The data showed that at the time of infection there was also CFU-E* in these animals, and all of these colonies from the DBA/2 mice and most of those from the CBA mice are epo dependent. At 1 wk after infection by FV-P, however, there was an increase in the number of epo-independent colonies in the marrows of both strains of mouse. This was then followed by a further increase in the number of CFU-E* colonies in the 2nd and 3rd wk after infection. Similar results were obtained in three independent experiments. These results showed that like the DBA/2 mice, there was also a significant increase in the levels of epo-independent CFU-E* in the CBA mice after infection with FV-P, indicating that the proportion of erythropoiesis under the control of epo after infection by FV-P was not responsible for the contrasting effects on the levels of RBC in the peripheral blood of these animals.

Pathology of Friend Disease in CBA and DBA/2 Mice Early after Infection with FV-P. We next examined the pathology of the Friend disease in CBA mice early after infection with FV-P. This was compared to the effects in the DBA/2 mice.

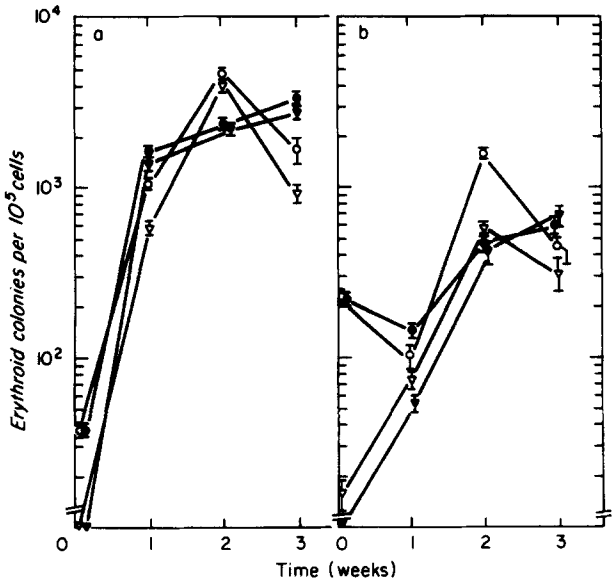

FIG. 4. Kinetics of change in CFU-E levels with or without added epo in the spleen and marrow of CBA and DBA/2 mice after infection of FV-P. 2×10^3 FFU of FV-P was injected into CBA or DBA/2 mice. At various times after infection, the number of CFU-E was measured in the spleens (a) or marrow (b) cells from these mice. ●, DBA/2 with 0.5 units of epo mice; ○, CBA mice with 0.5 units of epo; ▼, DBA/2 mice without epo; ▽, CBA mice without epo.

2×10^3 FFU of FV-P were injected into CBA and DBA/2 mice, and the kinetics and extent of splenomegaly were examined. Results in Fig. 5 showed that similar to the DBA/2 mice, a rapid splenomegaly was induced in CBA mice by FV-P. The extent of the splenomegaly in the CBA mice, however, was not as severe as in DBA/2 mice. Although the spleen weights of the DBA/2 mice reached a plateau of ~2–2.5 g, those of the CBA mice reached a maximum of ~1–1.5 g, one-half the size of the DBA/2 spleens.

The morphology of the cells in these enlarged spleens of CBA mice and DBA/2 mice were examined and compared. The cells from the spleens of both strains of mice were similar in morphology and included a large proportion of erythroblasts. The kinetics of changes in the proportion of erythroblasts was also monitored in the marrow and spleens of these DBA/2 and CBA mice. Fig. 6 shows the results of one such experiment. The percentage of erythroblasts decreased in the marrow 2 wk after infection before increasing at 3 wk (Fig. 6, upper panel). In contrast to these relatively small changes in the marrow, the erythroblasts increased dramatically in the spleens of these mice from ~10% to ~80–90% in 3 wk (Fig. 6, lower panel).

Erythroleukemia induction in susceptible mice by FV-A and FV-P is known to be associated with splenomegaly and changes in hematocrit values. These changes are probably related to the high mortality rate in these mice. To examine whether the erythroleukemias induced in CBA and DBA/2 mice are associated with different mortality rates after infection, 2×10^3 FFU of FV-P or FV-A were injected into CBA or DBA/2 mice, and the survival of these mice was monitored at 4 wk after infection. Results of these experiments are shown in Table II. The data indicate that significant differences in the mortality rates of these mice after infection with FV-A and FV-P

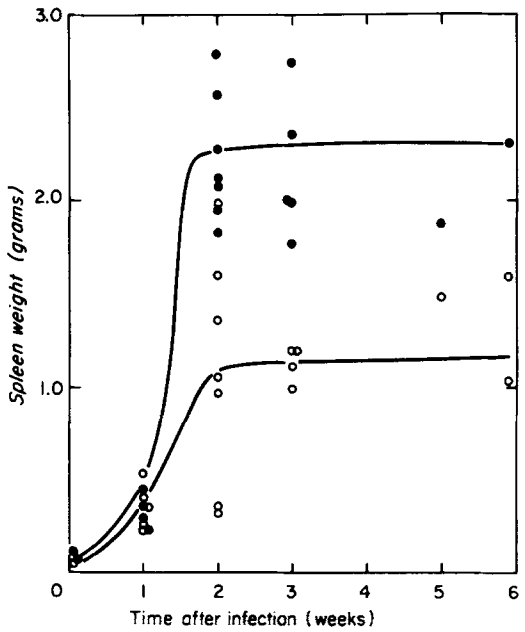

FIG. 5. Kinetics of change in spleen weights of CBA and DBA/2 mice after infection with FV-P. 2×10^3 FFU were injected into each CBA or DBA/2 mice. At various times after infection, the mice were killed and the spleen weights were determined. ●, DBA/2 mice; ○, CBA.

can be detected. The rates of mortality was higher in DBA/2 mice infected with FV-P and in CBA mice infected with FV-A.

Tumorigenic Colonies in CBA Mice Infected with FV-P. Recent results (13) have indicated that newly developed tumorigenic erythroid colonies (CFU-FV) capable of extensive proliferation can be detected in the spleen of DBA/2 mice late after infection with FLV. These in vitro colonies can be found in the spleens of these DBA/2 mice 3–6 wk after infection with FV-P and 10–12 wk after infection with FV-A (13, 15). To examine whether these tumorigenic cells can also be detected in CBA mice infected with FV-P and the relative rate of emergence of the CFU-FV, cells from spleens of CBA mice infected with FV-P were plated for CFU-FV 4–6 wk after infection. Results in Table III indicate that, similar to DBA/2 mice, tumorigenic cells, as detected by this assay, can also be found in the spleens of the CBA-infected mice. Furthermore, these colonies were detected at 4–6 wk after infection, indicating that the kinetics of emergence are similar to those in FV-P-infected DBA/2 mice.

Levels of Reticulocytes and in the Marrow and Spleens of DBA/2 and CBA Mice Infected with FV-P. The differences in the levels of RBC and reticulocytes in the peripheral blood of these infected mice (Fig. 1) could reflect the rates of erythropoiesis in their respective marrow and/or the spleens. The data presented above indicated that at the level of CFU-E* (Fig. 4) and erythroblasts (Fig. 6), the magnitudes and rates of increase of these progenitor cells were the same in the spleens and marrows of both strains of mice after infection with FV-P. The kinetics of changes of levels of reticulocytes in marrow and spleens were next examined. Results are illustrated in Fig. 7a and 7b. The data showed that there were rapid decreases in the percentages of reticulocytes in the marrows of both strains of mice from ~70–75% to ~20–25% after 2 or 3 wk

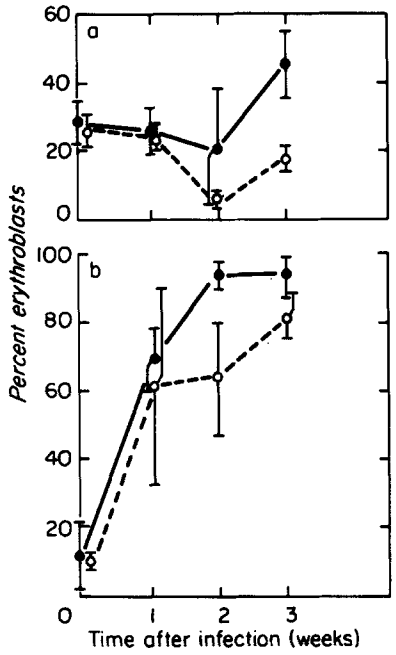

FIG. 6. Kinetics of change in the percent of erythroblasts in the marrow and spleens of CBA and DBA/2 mice infected with FV-P. 2×10^3 FFU were injected into each CBA or DBA/2 mice. At various times the mice were killed and the percent of erythroblasts was determined in the marrow (a) or spleens (b) of these mice. ●, DBA/2 mice; ○, CBA mice.

TABLE II

*Early Mortality of CBA and DBA/2 Mice Infected with FV-P or FV-A**

	Number of mice that died 4 wk after infection	
	FV-P	FV-A
DBA/2	53% (2/5; 3/5; 3/4; 2/5)	0% (0/5; 0/5; 0/4)
CBA	12% (1/4; 0/5; 1/4; 0/4; 0/4)	43% (2/5; 3/5; 2/6)

* CBA or DBA/2 mice were infected with 2×10^3 FFU of FV-P or FV-A. 4 wk after infection, the mortality of these infected mice was determined. Numbers in parentheses represent separate experiments.

(Fig. 7a). A difference in the percentage of reticulocytes in the spleens of DBA/2 and CBA mice, however, could be observed after infection with FV-P. Although the percentage of reticulocytes in the spleen of the infected CBA mice remained relatively constant at ~10% for 2 wk and then increased slightly to ~20%, the percentage of reticulocytes in the spleens of DBA/2 mice rose dramatically to a level of ~50% 2 wk after infection (Fig. 7b). Similar results were obtained in three separate experiments. This dramatic rate of increase in reticulocytes in DBA/2 mice with little change in the CBA mice reflects a higher rate of erythropoiesis in the spleens of the DBA/2 mice and might explain the difference in hematocrit values observed in these two strains of mouse.

Rate of Uptake of Radioiron in Femur and Spleens of DBA/2 and CBA Mice after Infection with FV-P. The rates of erythropoiesis as measured by uptake of ^{59}Fe in the femurs

TABLE III

*Detection of Tumorigenic Colonies (CFU-FV) in CBA and DBA/2 Mice Early and Late after Infection with FV-P**

	Number of tumorigenic colonies (per 5×10^6 spleen cells)	
	4–6 wk	*10–12 wk*
CBA mice infected with FV-P	12, 56	All mice died
DBA/2 mice infected with FV-P	62, 161	All mice died
DBA/2 mice infected with FV-A	0, 0	6, 82

* CBA or DBA mice were injected with 2×10^3 FFU each of FV-P. 4–6 wk or 10–12 wk after infection, the mice were killed, and the spleen cells were plated for CFU-FV, as described in Materials and Methods. FV-A were injected into DBA/2 mice as controls.

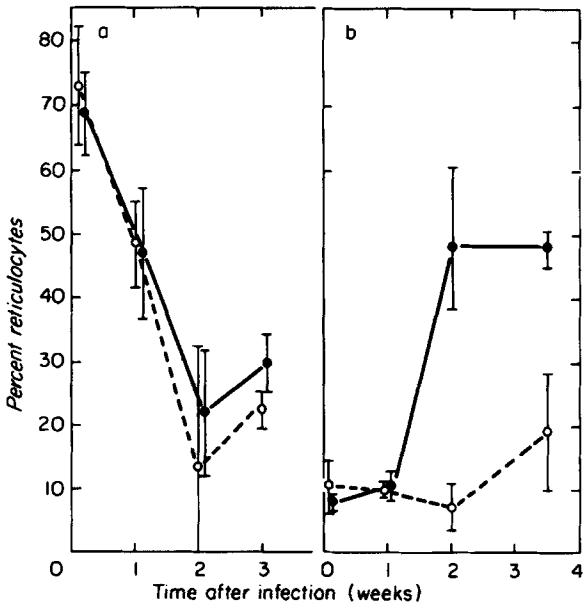

FIG. 7. Kinetics of change in the level of reticulocytes in the marrow (a) and spleens (b) of CBA or DBA/2 mice infected with FV-P. Same as Fig. 6, except reticulocytes were determined. ●, DBA/2 mice; ○, CBA mice.

and spleens of DBA/2 and CBA mice after infection of FV-P were also examined. At various times after infection with FV-P, ^{59}Fe in the form of $[^{59}Fe]Cl_3$ was injected intravenously into these mice, and the uptake into the femurs and spleens of these mice was measured and expressed as the percentage of ^{59}Fe injected. Results from these experiments are illustrated in Fig. 8a and 8b. The data showed that similar to the decrease in reticulocyte levels, a rapid drop in the rate of iron uptake was also detected in the bone marrows of the DBA/2 or CBA mice. In contrast, an increase in the rate of iron uptake was detected in the spleens of both strains of mouse. In addition, a significant difference in the rate of uptake was observed in the two strains of mice. While the rate of uptake of ^{59}Fe in the spleens of CBA mice was ~15%, the rate of uptake in the DBA/2 mice rose to 20%. These results support the above observation that there is a decrease in the level of erythropoiesis in the marrow and

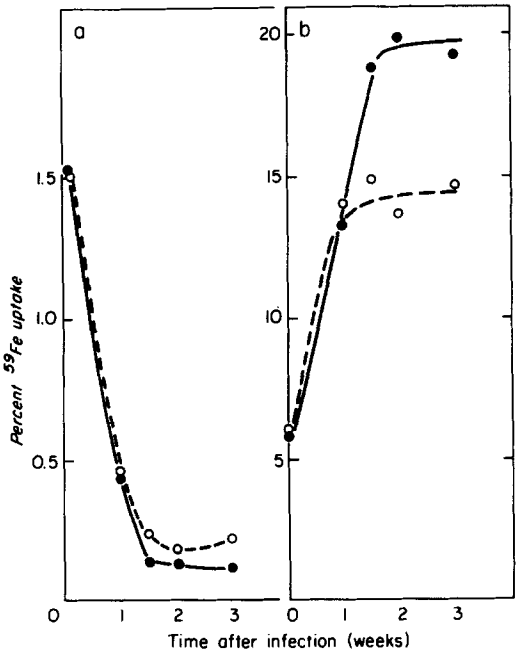

FIG. 8. Rate of iron uptake in marrow and spleens of CBA or DBA/2 mice infected with FV-P. 2 × 10^3 FFU of FV-P were injected into CBA or DBA/2 mice, and, at various times after infection, [^{59}Fe]Cl_3 was injected into these mice, and the rate of uptake of ^{59}Fe in the femurs (a) or spleens (b) was determined, as described in Materials and Methods, and expressed as percent of ^{59}Fe injected. ●, DBA/2 mice; O, CBA mice.

an increase in the level of erythropoiesis in the spleens of these mice shortly after infection with FV-P. Furthermore, the rate of erythropoiesis was higher in the DBA/2 mice.

Discussion

The pathological outcome after leukemia virus infection and transformation is the result of a complex array of interactions between viral genomes and the host. These developments occur at two levels, the cellular and the physiological. Effects at the latter level can be influenced by developments at the former. Examples of such viral-host interactions are probably best illustrated in the Friend leukemia virus (FLV) system, where a number of host genes are known to control the susceptibility of the host-to-FLV infections (26–32). The results presented in this communication indicate that, in addition to controlling the susceptibility, host genes are also important in determining the type of erythropoiesis induced by the FLV.

Much information is now available about the contributions made by the FLV viral genomes. Subsequent to infection by FLV complexes or F-MuLV, rapid changes in erythropoiesis can be detected. Concomitant with the splenomegaly, a rapid and dramatic elevation in the number of erythroid progenitor cells CFU-E* can be detected after infection of the FLV complexes (4, 16–18). After infection of newborn BALB/c mice by the helper-independent F-MuLV, however, a dramatic increase in the more immature erythroid progenitor bursts (BFU-E*) was observed instead of the increased CFU-E after FLV infection (33). These differences in erythropoietic response

are controlled by the viral determinants on the SFFV and F-MuLV genomes. Determinants within the two SFFV genomes ($SFFV_A$ and $SFFV_P$) have also been shown to be responsible for the differences in the erythroid populations in FV-A- and FV-P-infected mice. For example, erythroid colony-forming cells (CFU-E*) detected in susceptible mice early after infection with pseudo-types of $SFFV_P$ are independent of the hormone erythropoietin (16–18), whereas erythroid progenitor cells in $SFFV_A$-infected mice still require epo to proliferate (4, 19, 20).

After infection with FV-A, rapid anemia develops in the mice, whereas severe polycythemia is induced after infection with FV-P (1–5). The exact reason(s) for these contrasting physiological effects is not known. It is possible that the differences in the levels of RBC is accounted for by the nature of the epo-dependent and epo-independent erythropoiesis induced by the FV-A and FV-P, respectively. On the other hand, a variety of other effects, including the rates of hemolysis or cytolysis, rates of proliferation, or differentiation of the infected and transformed cells in the marrow and spleens of these animals, as well as the rates of transport of these erythroid cells to the blood, might also contribute to the final value of hematocrit. A study of these physiological changes after the infection of FV-A or FV-P has been described (34). It was suggested that the reasons behind the induction of polycythemia by FV-P and anemia by FV-A in the same strain of mouse might have been due to the increased rate of erythropoiesis after infection by FV-P and the expansion of blood volume after FV-A infection.

Data in this paper indicate that host genes are indeed involved in affecting the types of pathological changes in these FLV-infected mice. Unlike the host genes described before, which are known only to determine the susceptibility to infection by FLV (26–32), the host determinant(s) identified in this study controls the types of physiological alteration induced by the FLV complexes. A locus whose phenotypes segregate in a co-dominant nature in determining whether rapid anemia or polycythemia is induced in mice early after infection with FV-P has been identified (21), and it was proposed that this locus in the mouse be termed *Fv-5* (21). Mouse strains that develop anemia early after infection with FV-P have genotypes $Fv\text{-}5^{aa}$, whereas mouse strains with severe polycythemia have genotypes $Fv\text{-}5^{pp}$.

It has been established that Friend virus-induced erythroleukemia is a multistage disease (10–15) with well-defined early erythropoietic changes (1–5, 16–18) and late stages during which tumorigenic cell clones emerge (10–15). However, in addition to responding with different hematocrit values, mice infected with FV-A or FV-P or newborn mice infected with F-MuLV also respond differently with respect to some of these early and late changes (4, 33). The data in this communication indicate that although anemia is elicited, the early and late cellular aspects of the disease in $Fv\text{-}5^{aa}$ mice infected with FV-P are similar to those in $Fv\text{-}5^{PP}$ mice that develop polycythemia (16–18). First, like those of the FV-P-infected $Fv\text{-}5^{PP}$ mice, a dramatic increase in epo-independent erythroid progenitor cells, CFU-E*, was observed. Second, similar to the DBA/2 mice infected with FV-P, the CBA mice injected with FV-P also developed tumorigenic cell clones, as detected by the Friend-transformed cell colony assay (CFU-FV) (13, 14). In addition, the CFU-FV emerged at relatively early time (before 4–6 wk after infection), as compared with a minimum of 10 wk required for FV-A-infected mice (13) or the prolonged period (>15 wk) with RBC transfusion for F-MuLV-infected newborn mice (35, 36). From these cellular properties, it would appear that

the FV-P-injected CBA mice resemble the FV-P-infected *Fv-5*^{PP} mice rather than FV-A- or F-MuLV-infected mice. The development of anemia in these FV-P-infected *Fv-5*^{aa} animals might thus be the result of some other cellular or physiological functions.

In the experiments described in this communication, cellular and physiological alterations were examined. As would be expected, different levels of RBC and reticulocytes in the blood of the DBA/2 and CBA mice were observed, accounting for the contrasting changes in the hematocrits in the two strains of mouse. In the marrows of both strains of mice, a reduction of erythroblasts was noted early after infection. This was also accompanied by moderate increases in epo-independent CFU-E*, decrease in the levels of reticulocytes, and a reduction in the rate of hemoglobinization as measured by ^{59}Fe uptake. These viral-induced alterations, however, were similar in both strains of mice and thus cannot explain the differences in observed hematocrit values. Unlike the changes in the marrows, significant differences in erythropoietic activity were observed in the spleens of these two strains of mice. While the spleens of both strains of mouse contained about the same level of CFU-E* and erythroblasts, the proportion of reticulocytes in the spleens increased dramatically in DBA/2 mice to ~50%, while the reticulocyte level in the spleens of CBA mice remained unchanged at ~10–20%. In addition, the rate of erythropoiesis as measured by ^{59}Fe uptake also increased to a significantly higher level in DBA/2 mice. This higher rate of erythropoiesis combined with a more severe splenomegaly (2.5 g per DBA/2 spleen compared with ~1.25 g per CBA spleen) in the DBA/2 mice would result in a higher rate of production of RBC, accounting for the severe polycythemia. Thus, it would appear that the *Fv-5* locus might control the rate of proliferation of the erythroid precursors in the spleens of these infected animals. At the present time, it is not known which erythroid precursor cell or cells is controlled by this newly identified locus. However, because the levels of erythroid progenitor cell CFU-E* were similar in the two strains of mice, the cell or cells affected is probably more mature than CFU-E.

The identification of *Fv-5* adds another locus to a long list of host genes that are known to affect FLV-induced erythroleukemia and another mechanism by which these host genes affect the disease. Some of these genes, such as the *Steel* (37, 38), *W* (38, 39), *f* loci (40), and the *H-2* complex (41), are involved in controlling normal differentiation and developments. The best studied of this group is the *Steel* locus. It was determined (42) that this locus, which is known to prohibit the development of the hemopoietic stem cell, CFU-S (36), may also act by inhibiting the proliferation of the early transformed cells. The action of this locus, however, is specific for the early cells, as the late stage tumorigenic cells are immune from the control (12). The action of the other host determinants in the control of susceptibility to FLV is not so clear, although it is possible that the *H-2* complex may affect the disease via immune system (30) or by control of the expression of SFFV-specific sequences (27).

Of the other loci, *Fv-1* (43), *Fv-2* (29), and *Fv-4* (44), much is also known about the actions of the first two. *Fv-1* acts by inhibiting the replication of the helper F-MuLV, probably by controlling the integration of the provirus (45, 46). The *Fv-2* locus, on the other hand, affects the SFFV component and may act by inhibiting the expression of SFFV-specific sequences (27, 47). However, although it might appear that the *Fv-2* locus acts on the control or expression of viral sequences, it may only prevent infection of SFFV by controlling the rate of proliferation of the erythroid burst-

forming cells BFU-E (48). Thus, it is possible that the *Fv-2* locus and the *Fv-5* locus may both affect the Friend disease by controlling the rates of proliferation of erythroid precursor cells. In the case of the *F-2* locus, controlling the cycling of the earlier erythroid cells, BFU-E, could determine the susceptibility, as BFU-E might be the target cell (48). On the other hand, control of the proliferation of more mature erythroid cells as in *Fv-5* might only determine the severity of the disease as infection is established in a less mature erythroid cell. Thus, the higher rate of proliferation of the infected cells in the $Fv\text{-}5^{PP}$ mice may affect only the type of erythropoiesis and the mortalities of these mice, but not susceptibility to infection by the virus.

Summary

This report confirms that the *Fv-5* locus controls the types of erythropoiesis induced by Friend erythroleukemia virus (FLV) (21) and extends the study to investigate the mode of action of this locus. With the use of FLV obtained by a variety of procedures, we showed that the polycythemia spleen focus-forming component ($SFFV_p$) was responsible for the contrasting changes of hematocrits observed in FV-P_p (polycythemia strain)-infected DBA/2 ($Fv\text{-}5^{pp}$) or CBA ($Fv\text{-}5^{aa}$) mice. These changes in hematocrits were found to be a direct result of the rise in circulating reticulocytes and erythrocytes in DBA/2 mice and a corresponding drop of these erythroid cells in CBA mice 2 wk after infection. Examination of the FV-P-induced cellular changes indicated that dramatic increase in erythropoietin (epo)-independent erythroid precursor (CFU-E*) cells was detected in the spleens and marrow of both strains of mice. The epo responsiveness of the CFU-E in the uninfected and FV-P-infected CBA and DBA/2 mice was also very similar. Similar to FLV-infected DBA/2 mice, the FV-P-infected CBA mice also developed tumorogenic cells (CFU-FV) relatively early after infection (4–6 wk). Study of the physiological and pathological changes in the marrows and spleens of these infected mice indicated that significant differences were found in the spleens of the two strains of mice. The percent of reticulocytes in the spleen cells of CBA mice remained between 10 and 20%, and level of the DBA/2 mice increased to ~50%. This higher rate of erythropoiesis was also reflected in the significantly higher rate of uptake of ^{59}Fe in the spleens of the DBA/2 mice. These results suggest that the *Fv-5* locus might control the hematocrit levels of these mice by regulating the rates of erythropoiesis in the spleen levels of these mice, probably by affecting the rate of proliferation of an erythroid cell or cells. The erythroid cell(s) affected is likely to be more mature than the erythroid progenitor, CFU-E, as the levels of CFU-E in these two strains of mice were similar. The hypothesis that *Fv-5* may control the rates of proliferation of a late erythroid (cell(s) is also supported by the significantly higher spleen weights found in the infected DBA/2 (~2.5 g/spleen) mice than in the CBA (~1 g/spleen) strain.

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