# MODULATION OF ERYTHROPOIESIS BY THE HELPER-INDEPENDENT FRIEND LEUKEMIA VIRUS F-MuLV*

BY YOSHIYUKI NIHO,‡ TSUNEFUMI SHIBUYA, AND TAK W. MAK§

*From the Ontario Cancer Institute; and the Department of Medical Biophysics and Institute of Medical Sciences, University of Toronto, Toronto, Canada M4X 1K9*

Retroviruses are ubiquitous agents present in wild mice as well as inbred laboratory mice. They occur as endogenous proviruses and are transmitted vertically through the germ line cells (1, 2). Although they are present in the DNA of all the cells in the form of integrated provirus, their regulation is under strict control and they are strongly repressed in most tissues. These viruses, however, can be expressed naturally late in life in mice with certain genetic dispositions (3, 4). Their expression, though, is under genetic and developmental control and is often confined to the hemopoietic tissues, notably the cells in the bone marrow, spleen, and the thymus (2, 5–7). The expression of these viruses and their genes in these mice in some cases is associated with a variety of hemopoietic disorders such as autoimmune disease (8), lymphomas, and leukemias (3, 4). In some instances, infectious retroviruses have been recovered from these diseased animals, and these are capable of inducing hemopoietic aberrations similar to those in the diseased mice from which the viruses were isolated (5, 9–11). Viral induced modification of hemopoietic differentiation is thus well established.

To understand the relationship between normal and virally induced hemopoietic differentiation and the genetic control of these processes, much effort has been devoted to the in vitro and in vivo studies of these events. The best-studied viral modified hemopoietic system is probably Friend erythroleukemia. Friend erythroleukemia virus (FLV),[1] isolated in 1957 by Charlotte Friend (9), is a complex of two viruses, a replication-defective, erythroleukemia-inducing, spleen focus-forming virus (SFFV) (12) and a replication-independent leukemia virus (F-MuLV) that provides the necessary function for SFFV to complete its replicative cycle (13, 14). Subsequent to infection with FLV, susceptible adult and newborn mice develop very rapid (1–3 wk) erythropoietic changes including splenomegaly (9, 15) and development of anemia or polycythemia, depending on whether the anemia strain (FV-A) (9) or the polycythemia strain (FV-P) (16) is used. This induction of early polycythemia by FV-P, however, has recently been shown to be mouse strain dependent (16), for whereas severe polycythemia is induced early in the mouse strains DBA/2, BALB/c, and AKR, rapid anemia is induced in mouse strains CBA and C3H (16).

[1] *Abbreviations used in this paper:* BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; DMSO, dimethyl sulfoxide; epo, erythropoietin; FLC, Friend erythroleukemic cell; FLV, Friend erythroleukemia virus; F-MuLV, replication-competent virus in FLV; FV-A, FV-P, anemia and polycythemia strain of FLV; PHZ, phenylhydrazine; SFFV, spleen focus-forming virus.

In addition to pathological changes, the FLV complexes also induce rapid hemo-poietic changes, notably in the progenitor cells in the erythroid pathways. The most dramatic change is the increase in colony-forming unit-erythroids $(CFU-E*)^2$ (17–19). In addition, differences in the nature and frequencies of erythroid progenitor cells can also be detected in mice infected with the two strains of FLV. For example, whereas the proliferation and differentiation of CFU-E* in FV-A-infected mice are still under the control of erythropoietin (epo) (19, 20), erythropoiesis and development of CFU-E* in FV-P-infected mice are independent of epo (17–19, 21, 22). These rapid erythropoietic modulations are functions of the SFFV components (19), and little change can be detected when the helper virus F-MuLV alone is injected into adult susceptible mice.

Several reports (20, 23–25), however, indicate that when newborn BALB/c or NIH Swiss mice are used, the replication-independent F-MuLV, which has often been considered's simply the helper virus, can also induce splenomegaly. In this study, we examinedthe modulation of erythropoiesis by the replication-independent F-MuLV in newborn and adult mice.

## Materials and Methods

*Virus.* An NB-tropic Friend F-MuLV was cloned from a FV-P complex by limiting dilution (14). This virus has been shown repeatedly over the last 5 yr (14, 19, 20, 26) by both biochemical and biological methods to contain no detectable SFFV. Titer of the F-MuLV was determined by the XC plaque assay (27).

*Mice.* BALB/c and DBA/2 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Newborn BALB/c mice were obtained from the Ontario Cancer Institute Animal Colony.

### Culture of Progenitor Cell Colonies

CFU-E AND ERYTHROID BURST (BFU-E) ASSAY.    CFU-E and BFU-E progenitor colony assays were performed as described previously (19, 28). In brief, 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 or Iscove's modified Dulbecco's medium supple-mented with $7.5 \times 10^{-5}$ M 2-mercaptoethanol, 1% deionized bovine serum albumin, and 30% fetal calf serum (Flow Laboratories, Inc., Rockville, MD). Erythropoietin (step III, lot 3038; Connaught Laboratory) was added at a final concentration of 0.2–2.0 U/ml for CFU-E or for BFU-E colony assays. Colonies containing 8–64 hemoglobinized cells at day 2 were counted as CFU-E or CFU-E*, and clusters of three or more colonies were counted as BFU-E or BFU-E* at day 7.

## Results

To examine changes in the hemopoietic progenitor cells, 1-d-old BALB/c mice were infected with $3 \times 10^5$ XC plaque U of F-MuLV. 7 wk after infection, the spleens and marrow cells were obtained from these infected mice and the frequencies of CFU-E* and BFU-E* were measured. These results were compared with those obtained from uninfected BALB/c mice. Results in Table I indicate that whereas the levels of CFU-E and BFU-E in the spleens and marrows of the uninfected mice are consistent with those reported previously for normal mice, changes in the erythroid bursts colonies can be detected in the spleens and marrows of the mice infected by F-MuLV.

Unlike mice infected with the FLV complexes, BALB/c mice infected by F-MuLV

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[2] CFU-E and BFU-E induced by infection with virus are denoted as CFU-E* and BFU-E*, respectively.

TABLE I

*BFU-E and CFU-E in Spleens and Marrows of Uninfected BALB/c Mice and BALB/c Mice Infected with F-MuLV as Newborns*

|  |  | CFU-E/$10^5$ cells | | BFU-E/$10^5$ cells | |
|---|---|---|---|---|---|
|  |  | −epo | +0.5 epo | −epo | +2.0 epo |
| Uninfected | Marrow | 0 | 141.3 ± 2.5 | 0 | 7 ± 0.8 |
|  | Spleen | 0 | 36.7 ± 3.8 | 0 | 2.9 ± 0.8 |
|  |  | CFU-E*/$10^5$ cells | | BFU-E*/$10^5$ cells | |
| Infected as newborn | Marrow | 0 | 114.7 ± 13.5 | 0 | 223 ± 50 |
| with F-MuLV | Spleen | 0 | 16 ± 3.5 | 0 | 1258 ± 121 |

Spleen and marrow cells were obtained from normal 7-wk-old BALB/c mice or 7-wk-old BALB/c mice infected with 3 × $10^5$ XC plaque U of F-MuLV at the time of birth. The cells were washed and plated for CFU-E or CFU-E* and BFU-E or BFU-E* as described in Materials and Methods. CFU-E* and BFU-E* denote CFU-E and BFU-E-like colonies in the virally infected animals. Data are expressed as number of colonies per $10^5$ nucleated cells. At least four plates were used in each determination.

as newborns did not have dramatic increase in the level of either epo-dependent or -independent CFU-E* in the spleen or marrow (possibly even a slight decrease). Instead, there was a dramatic increase in the levels of BFU-E-like colonies. This level, which reached ~1,500 bursts/$10^5$ cells or ~5 × $10^7$ BFU-E*/spleen was much higher than those reported (29) for mice infected with the FLV complexes (T. Shibuya and T. W. Mak, unpublished observations). A similar picture was seen in the bone marrow of F-MuLV-infected mice, with ~300 BFU-E*/$10^5$ cells or 3 × $10^5$ cells/femur. These F-MuLV-induced BFU-E* colonies were similar in size and morphology to the BFU-E colonies in uninfected animals (Fig. 1). However, they differ from the normal BFU-E in that they contained less hemoglobinized cells per colony. To demonstrate that these BFU-E* were erythroid in nature, 20 colonies were picked and stained for spectrin-positive cells by immunofluorescence as described previously (24). Results showed that >99% of the cells contained the erythroid-specific marker spectrin, confirming the erythroid nature of these BFU-E*. Although a high level of erythroid bursts (200–300 BFU-E*/$10^5$ cells) were observed, the number of CFU-E*/$10^5$ cells in the marrow and spleens was unchanged or slightly reduced. The formation of these erythropoietic progenitor cells was dependent on epo, as no erythroid colonies or erythroid bursts could be detected in the absence of the hormone.

*Epo Requirement in the Erythroid Progenitor Colonies (CFU-E and BFU-E) in F-MuLV-infected Mice.* The level of epo required for the formation of erythroid progenitor colonies BFU-E* and CFU-E* in mice infected with F-MuLV was also examined. Cells from the marrow and spleens of infected animals were obtained at 7 wk after infection, and the levels of CFU-E* and BFU-E* were determined in the absence of any added epo and in the presence of varying amounts of added epo. The results of such experiments are illustrated in Figs. 2 and 3. As in the results described above, the spleen cells contained a high level of erythroid bursts and their formation was dependent on added epo. The data in Fig. 2 show that the number of BFU-E* increased with added epo and reached a plateau of ~1,200–1,500 BFU-E*/$10^5$ spleen cells at 0.2 U of epo/plate and ~500 BFU-E*/$10^5$ marrow cells at ~1.0 U/plate of the hormone. The epo dose-response curves for the formation of the CFU-E* in the
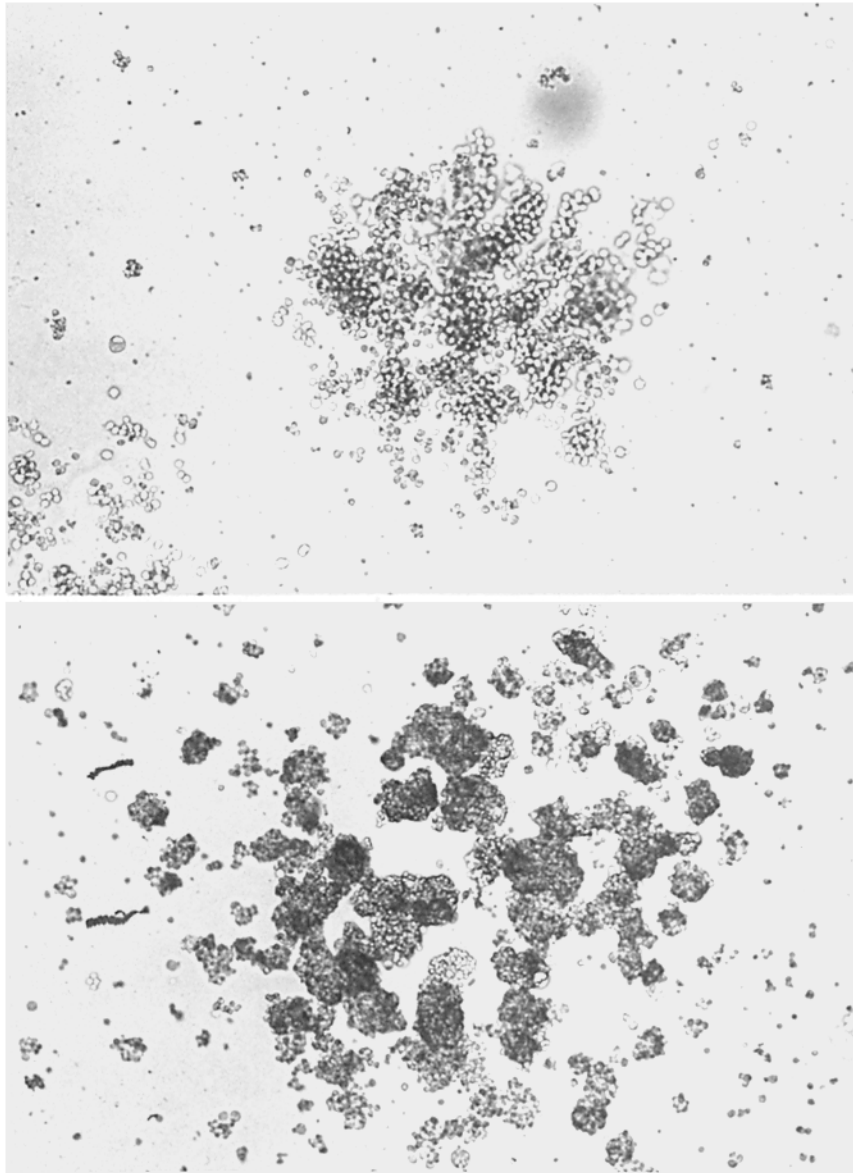
Fig. 1. Colony morphology of erythroid bursts from spleens of BALB/c mice infected with F-MuLV as newborn or uninfected BALB/c mice. Spleen cells were obtained from 7-wk-old BALB/c mice infected with $3 \times 10^5$ XC plaque U of F-MuLV (upper panel) or uninfected BALB/c mice (lower panel) and cultured for erythroid bursts in the presence of 2.0 U/plate of epo as described in Materials and Methods.

F-MuLV-infected spleen and marrow cells are illustrated in Fig. 3. As can be seen, the formation of CFU-E* in the spleen and marrow cells in mice infected with F-MuLV as newborns was also dependent on the addition of epo. Similar to the results above, the number of CFU-E* in the F-MuLV-infected spleen cells was low, reaching a level of ~50 CFU-E* at as low as 0.2 U of epo/plate. The level of CFU-E* in the
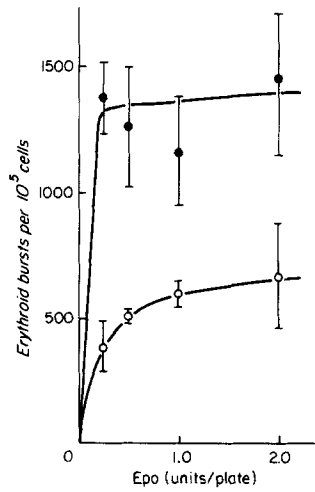
Fig. 2.    Epo dose-response curves of BFU-E* from the spleens and marrow cells of BALB/c mice infected at birth with F-MuLV. BALB/c mice were infected at birth with $3 \times 10^5$ XC plaque U of F-MuLV. 7 wk after infection, the mice were killed and the spleen cells (●) and marrow cells (○) plated for BFU-E in $\alpha$-medium at 0, 0.2, 0.5, 1.0, and 2.0 U of epo per plate.


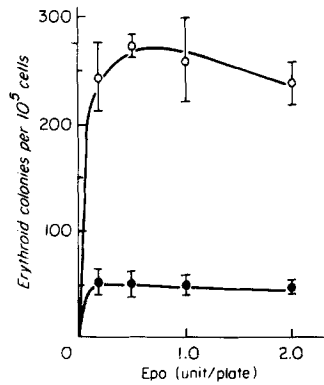
Fig. 3.    Epo dose-response curves for CFU-E* in BALB/c mice infected at birth with F-MuLV. Spleen cells (●) and marrow cells (○) were obtained as described in Fig. 2, and CFU-E* were measured at 0, 0.2, 0.5, 1.0, and 2.0 U of epo/plate. In this experiment, Iscove's modified Dulbecco's medium was used.

marrow cells increased to a plateau of ~250 CFU-E*/$10^5$ cells at ~0.5 U of epo/plate. For comparison purposes, dose-resonse curves for BFU-E and CFU-E in normal uninfected BALB/c mouse marrow are also summarized in Fig. 4.

*Modulation of Erythropoiesis in Adult Mice by F-MuLV and Phenylhydrazine (PHZ).* Results described above and elsewhere (13, 20, 30) indicate that injection of F-MuLV alone into adult mice results in little or no erythropoietic changes or splenomegaly. Modulation of the erythropoietic changes in adult mice, however, can be induced by the addition of certain chemicals like PHZ. Furthermore, Tambourin et al. (31) have shown that administering the polycythemia FLV complex in conjunction with PHZ resulted in an accelerated disease. In a study similar to those described by S. Ruscetti and E. Scolnick (unpublished observation), we have examined
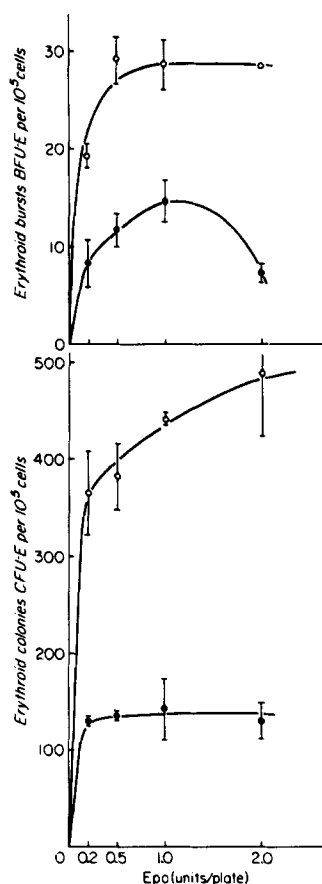
Fig. 4. Epo dose-response curves for BFU-E and CFU-E in uninfected BALB/c mice. BALB/c mice at 7 wk old were killed, and the bone marrow cells were plated for BFU-E (upper panel) or CFU-E (lower panel) in the presence of either Iscove's modified Dulbecco's medium (○) or α-medium (●) as described in Materials and Methods.

whether F-MuLV can affect the erythropoiesis changes in adult mice treated with PHZ.

To this end, adult mice (8 wk old) were injected with one of the following: (a) 1.5 × 10⁶ XC plaque U of cloned F-MuLV alone (time 0); (b) 40 µg PHZ/gram at the following times: 1 and 2 d before time 0; 1, 2, 3, 8, 12, 16, 20, 22, and 26 d after time 0; (c) 1.5 × 10⁶ XC U of F-MuLV at time 0 with PHZ regiment as in (b); (d) 2 × 10³ SFFV U of FV-P and the PHZ regiment at times −2, −1, 1, 2, and 3 d with respect to time 0. At various times after the administration of these viruses and PHZ, the spleen weights, hematocrits, and number of CFU-E in the spleens of of these mice were measured. Results of these experiments are summarized in Fig. 5. As can be seen in Fig. 5 (upper panel), only a sight increase in spleen weights could be detected in these adult mice infected with F-MuLV alone. With the administration of PHZ there was a moderate increase in spleen weights from ~0.1 to ~0.3 g/spleen. This slight splenomegaly was maintained until the administration of PHZ ceased at 4 wk. When
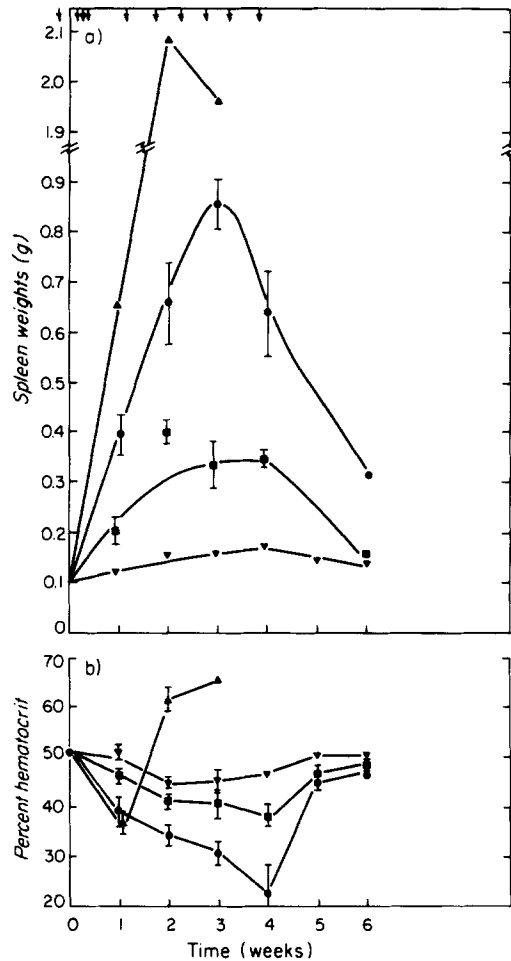
Fig. 5.   Modulation of spleen weights and hematocrits of adult BALB/c mice with F-MuLV, PHZ, F-MuLV with PHZ, or FV-P with PHZ. 8-wk-old BALB/c mice were injected with the following: (a) 1.5 × 10⁶ XC-plaque U of F-MuLV (▼); (b) 40 μg/g PHZ at the times indicated by arrows at the top of the figure (■); (c) F-MuLV with PHZ as in (b) (●); (d) 2 × 10³ SFFV U of FV-P with PHZ as in (b) (▲). At weekly intervals, the spleen weights (upper panel); or hematocrits (lower panel) of these mice were measured.

the F-MuLV was injected in conjunction with the administration of PHZ, however, a dramatic increase in spleen weight up to ~1 g/spleen was detected by 3 wk, indicating that the F-MuLV could increase the splenomegaly of PHZ-treated mice. Mice infected with FV-P along with PHZ as controls also developed splenomegaly.

The kinetics of changes in hematocrit values of mice given the different treatments are illustrated in Fig. 5, lower panel. Results showed that there were only slight changes in the hematocrit values in adult mice infected with F-MuLV. With the administration of PHZ, there was a moderate drop in the level of hematocrits to ~40% at 4 wk after administration of the chemical. However, with the injection of F-MuLV and PHZ, there was a gradual decrease in the level of hematocrit to ~25% by 4 wk. This level of hematocrit recovered to normal values only after the adminis-
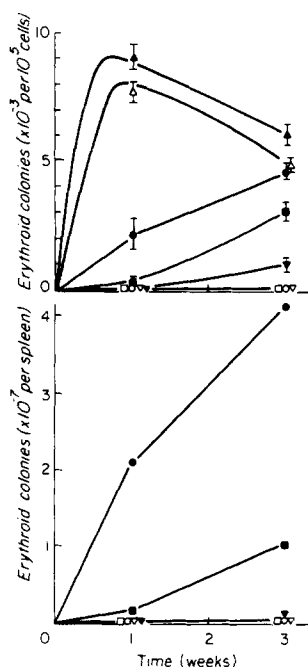
Fig. 6.  CFU-E* in adult BALB/c mice injected with F-MuLV, PHZ, F-MuLV with PHZ, or FV-P with PHZ. 8-wk-old BALB/c mice were injected with F-MuLV (▼, ∇), PHZ (■, □); F-MuLV with PHZ (●, ○); or FV-P with PHZ (▲, △) as described in Fig. 5. At 1 and 3 wk after administrations of these statements, the spleen cells of these mice were plated for CFU-E* in the presence (solid symbols) or absence (open symbols) of 0.5 U of epo/plate. The results are expressed as the number of CFU-E* per $10^5$ nucleated cells (upper panel) or number of CFU-E* per spleen (lower panel).

tration of PHZ ceased. With the administration of PHZ and FV-P, the hematocrit values dropped after 1 wk, but reversed and rose to a level of ~65% by 3 wk. The levels of erythroid progenitor cells in these adult mice under the different treatments were also monitored.

The number of CFU-E* in the spleens of these mice was measured before infection and at 1 and 3 wk after initiation of infection. Results in Fig. 6, upper and lower panels, indicate that with the injection of F-MuLV there was a moderate increase in the number of CFU-E*. All the CFU-E* were epo dependent. With the administration of PHZ, the level of CFU-E* increased to ~3,000/$10^5$ spleen cells or $10^7$ per spleen 3 wk later. The majority of these erythroid progenitor cells were also dependent on the addition of epo. The level of CFU-E*, however, increased further when F-MuLV and PHZ were administered. These erythroid colonies, which were also epo-dependent, increased to ~4,500/$10^5$ cells or ~4 × $10^7$ CFU-E*/spleen. When FV-P and PHZ were injected, there was a dramatic increase in the levels of CFU-E* in the spleens. These CFU-E* were, however, independent of epo. Experiments similar to those described here with F-MuLV and PHZ were also performed in adult (8 wk old) DBA/2 mice. Results indicated that like those in BALB/c mice, the injection of F-MuLV into PHZ treated mice also induced splenomegaly, a decrease in hematocrits, and enhancement of levels of CFU-E*.

## Discussion

The present study confirms and extends earlier findings (20, 23–25) that F-MuLV, which is often presumed not to play an important role in Friend disease, can induce erythropoietic changes in newborn BALB/c mice. In addition, it was shown that if the hemopoietic component was perturbed by PHZ, F-MuLV can also modulate erythropoiesis in adult mice. The erythropoietic pertubations by F-MuLV in newborn mice, however, are different from those described before for the infection by the FLV complexes. It was reported that subsequent to infection by FLV complexes, a dramatic increase (up to 5,000-fold/spleen) in CFU-E* was observed (17–22). In addition, it was determined that the nature of these CFU-E* in the FV-A- or FV-P-infected spleens was different. Whereas the proliferation and differentiation of CFU-E* in FV-A-infected mice are still under the control of epo (19, 20), erythropoiesis and development of CFU-E* in FV-P-infected mice are independent of the hormone (17–19, 21, 22). It was reported that changes in the erythroid bursts can also be detected in response of FLV infection both in vivo (29) and in vitro (32, 33). However, as noted in these mice infected in vivo by the FLV, the enhancement of the erythroid bursts was insignificant when compared with the dramatic increase in CFU-E*. Although the CFU-E* increase in the FLV-infected spleens can be as much as 5,000 times/spleen, the elevation in BFU-E* in the infected spleens was only ~20–30-fold/spleen (28, 29). This increase in BFU-E* could be partially accounted for by the dramatic increase in spleen weights of these mice.

In the present study, we have shown that subsequent to F-MuLV infection in newborn BALB/c mice, a very different picture was observed. Instead of the increase in CFU-E* observed in FLV-infected mice, a dramatic elevation in the number of BFU-E* was observed in the spleens and marrows of mice infected by F-MuLV as newborns. This increase of BFU-E* in the spleens can reach a level of ~3,000 times that of the normal BFU-E in uninfected spleens. The number of CFU-E* however, did not increase in these F-MuLV-infected mice. This finding suggests that the epo-dependent erythroid colonies observed earlier (20) were BFU-E* and not CFU-E*. It is possible that because the erythroid colonies in the earlier report (20) were evaluated only on day two, the colonies scored were those destined to be BFU-E*.

Although these F-MuLV BFU-E* resemble the normal bursts, it is not clear how similar they are to the normal day 7 BFU-E reported by Axelrad et al. (34) or to the erythroid bursts induced by FLV complexes in vivo (29) and in vitro (32, 33). At least one minor difference from the normal BFU-E can be detected. When compared with the normal BFU-E under the experimental conditions described here, these F-MuLV-induced BFU-E* colonies contained less hemoglobinized cells.

At this time it is not clear why F-MuLV can induce these erythropoietic changes in newborn BALB/c mice but not in adults. Ruscetti et al. (35) have recently suggested that the ability to induce these erythropoietic changes may be related to the release of a virus, termed mink cell focus-forming virus. Another reason may be related to the nature of the hemopoietic compartment of the newborn mice, which is known to be more active, and different from that of adult mice (36). The possibility that a more active hemopoietic compartment may be more susceptible to infection and modulation by F-MuLV is also supported by our findings. We have shown that if the hemopoietic compartment of adult mice is perturbed by treatment with PHZ, splenomegaly can be induced by infection with F-MuLV. This finding was first observed by S. Ruscetti,

T. Feild, L. Davis, and A. Oliff (unpublished observations). In the present study we have confirmed this finding and extended it to other erythropoietic changes, including the induction of anemia and the enhancement of CFU-E*. It is possible that after treatment with PHZ, a chemical known to induce anemia (31), the hemopoietic tissues will regenerate and the immature hemopoietic cells, including the erythroid precursor cells, will cycle more actively. This cycling of the erythroid precursor cells may render the mouse more susceptible to infection by F-MuLV. The hypothesis that cycling of cells may be important for infection and transformation has previously been proposed, first by Humphries and Temin (38) and more recently by Suzuki and Axelrad (36) and Oliff et al. (38).

On the basis of data reported previously (20, 23–25) and this communication, it is not possible to determine the target cell(s) for infection or transformation by F-MuLV, nor is it possible to determine the relative stage of differentiation of these F-MuLV-transformed cells with respect to those induced by the FV-A or FV-P complexes. Based on the finding herein that a dramatic enhancement of BFU-E* was observed in these F-MuLV-infected spleens with no increase in the levels of the more mature CFU-E*, as in the case of the FV-A- and FV-P-infected mice (17–20), it is tempting to postulate that the F-MuLV-transformed cells are less differentiated than those from the FV-A- or FV-P-infected spleens. Support of this hypothesis can also be found in the study of the recently established Friend erythroleukemic cell (FLC) lines from the F-MuLV-infected spleens (24, 38). It was determined that whereas FLC lines established from FV-P-infected spleens contain a high level of spectrin- and a low level of benzidine-positive cells (39), FLC lines from F-MuLV-infected newborn mice contain only spectrin-positive cells, but no benzidine-positive cells (24). Furthermore, it was found that although FLC lines established from FV-A and FV-P infected spleens respond to epo or dimethylsulphoxide (DMSO) to differentiate along the erythroid pathway (39), FLC lines from F-MuLV-infected mice will not respond to induction by DMSO (24, 40) epo (24), or other commonly used inducers (38). The finding that a dramatic elevation of BFU-E* with no increase in CFU-E* also suggests that the leukemic block induced by F-MuLV infection may be between that of the BFU-E and CFU-E. Furthermore, these data would suggest that the target cell(s) may be at the level of BFU-E or earlier. Alternatively, F-MuLV may infect and/or transform cells that in turn release or induce factors that can affect a more immature erythroid subpopulation(s).

## Summary

Friend leukemia virus (FLV) is a complex of two viruses, a defective spleen focus-forming component (SFFV) and a helper-independent virus, F-MuLV. Although the effects of the erythropoietic changes in susceptible adult mice after infection of FLV have been attributed to the SFFV component, it has been shown that the F-MuLV alone can induce certain erythropoietic changes in susceptible newborn mice. In the present study, we have shown that very different erythropoietic changes were observed after infection with F-MuLV. Unlike the FLV complexes, the F-MuLV did not induce in newborn BALB/c mice an increase in the erythroid progenitor cells (CFU-E*). Instead, a dramatic increase in the bursts (BFU-E*) can be detected in the infected spleens and marrows. The frequency of BFU-E* in the infected spleens increased to a level of ~1,500/$10^5$ cells or 5 × $10^7$/spleen. In the marrows, frequency

increased to a level of 300 BFU-E*/$10^5$ cells or $\sim$3 $\times$ $10^5$/femur. These F-MuLV-induced bursts, BFU-E*, differ from the BFU-E in uninfected mice. For example, although the morphology of the erythroid bursts in the F-MuLV infected mice was similar to that in the uninfected mice, the colonies were less hemoglobinized when compared with those in the uninfected mice.

We have also shown that if adult BALB/c or DBA/2 mice were administered phenylhydrazine (PHZ), infection of cloned F-MuLV can induce certain erythropoietic changes. For example, although F-MuLV alone has little effect on the spleen weights (0.1–0.15 g) of adult mice and PHZ can only moderately increase the spleen weights (0.3 g), infection of F-MuLV in PHZ-treated mice can induce a severe splenomegaly ($\sim$1 g). Similarly, the infection of F-MuLV in PHZ-treated mice can also induce a severe anemia and an increase in the frequency of CFU-E*.

These results indicate that F-MuLV, without the SFFV components, can modulate erythropoesis in newborn and adult mice. The finding that F-MuLV in newborn BALB/c mice elicit very different erythropoietic changes also has interesting implications. The induction of a dramatic increase in BFU-E* with no increase (possibly even a slight decrease) in CFU-E* implies that the leukemic block in F-MuLV-infected newborn mice is at a stage between BFU-E and CFU-E. The data also suggest that F-MuLV infect different, possibly more immature, erythroid target cells than the FLV complexes.

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