Increased Hematopoiesis in Mice Soon after Infection by Friend Murine Leukemia Virus

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Friend murine leukemia virus (F-MuLV), an erythroleukemogenic replication-competent retrovirus, induces leukemia in its host after a long latency. However, the early effects of infection may determine the pathway that eventually leads to malignant transformation. To determine how F-MuLV affects host cell proliferation soon after infection, BALB/c mice were inoculated with virus and then were assayed for susceptibility to appropriately pseudotyped spleen focus-forming virus (SFFV) as an indicator of erythropoietic activity. Twelve-week-old mice exposed to F-MuLV for 9 days were more susceptible (by a factor of 30) to superinfection by SFFV than were nonviremic mice. To test whether increased susceptibility was the result of increased hematopoietic activity, hematopoietic progenitors from the spleens of F-MuLV-infected mice were enumerated with a clonal culture assay. Nine days after inoculation with F-MuLV, the numbers of colony-forming progenitors increased by a factor of 4. Morphological analysis of the cultured colonies showed that erythroid, granulocytic, monocytic, and mixed granulocytic-monocytic progenitors all had increased. Thus, F-MuLV more rapidly induced a generalized increase in hematopoiesis than has previously been reported. The splenic hyperplasia induced by F-MuLV soon after infection may explain its ability to accelerate leukemogenesis in mice also infected by the polytropic Friend mink cell focus-forming virus.

The genome of spleen focus-forming virus (SFFV) encodes the virally derived oncogene *gp55* (1), which induces the proliferation of cells expressing the receptor for erythropoietin (15). The target cell for transformation by SFFV is the mature burst-forming unit of the erythroid lineage (13). Inoculation of susceptible mice with SFFV causes a massive and localized expansion of the erythroid compartment, such that macroscopic foci are evident 9 days after infection, with rapid progression to malignancy (2, 8).

Because SFFV is replication defective, it is infectious only if accompanied by a helper virus which supplies viral structural proteins. The cellular receptor specificity of an SFFV virion is therefore determined by the envelope glycoprotein encoded by its helper virus, making it a useful indicator of viral susceptibility in mice. Cells infected by a retrovirus using one cellular receptor are susceptible to superinfection by retroviruses only using a distinct receptor for entry (29, 30); interference established in cultured cells by an ecotropic murine leukemia virus (MuLV) to ecotropic virus challenge, for example, can be overcome by superinfection with amphotropic, dualtropic, or xenotropic viruses (as described in reference 23). Accordingly, mice preinfected with ecotropic Friend MuLV (F-MuLV) were resistant to superinfection by SFFV when SFFV was packaged by F-MuLV but were susceptible to superinfection by amphotropic SFFV, as measured by spleen focus-forming activity (20). The susceptibility of F-MuLV-infected mice to superinfection by amphotropic SFFV was significantly greater than the susceptibility of mice free of virus at the time of challenge, leading to speculation that F-MuLV infection influenced the erythroid cells susceptible to transformation by SFFV. F-MuLV inoculation might increase susceptibility to SFFV if it were to increase the number of mature burst-forming units of the erythroid lineage available for infection. To test this hypothesis, mice were inoculated with F-MuLV and assayed for hematopoietic activity by challenge infection with SFFV or

by culturing of spleen cells in a clonal assay for committed hematopoietic progenitors.

BALB/cByJ mice purchased from Jackson Laboratory (Bar Harbor, Maine) or bred at McArdle Laboratory were used to measure susceptibility to SFFV challenge after inoculation with F-MuLV. During infection, mice of the BALB/c strain fail to mount an antiviral antibody response to SFFV(F-MuLV) (5) and do not activate the class I major histocompatibility complex-restricted effector cells thought to be responsible for the rejection of SFFV-infected target cells in resistant strains of mice (6).

The F-MuLV pools used in these experiments were produced by transfecting NIH 3T3 cells with clone 57 (22) proviral DNA by the calcium phosphate precipitation technique described by Hopkins et al. (12), culturing the transfected cells in Dulbecco's modified Eagle medium containing 5% calf serum (Hyclone), and passaging them for at least 2 weeks. Virus stocks were collected in the same culture medium 24 h after feeding and were frozen at -70°C . F-MuLV stocks were assayed by the UV-XC plaque-forming assay (24): NIH 3T3 cells were exposed to virus, cultured for 5 to 6 days, UV irradiated, and then overlaid with rat XC cells for 3 days before syncytial plaques were scored. Mice inoculated with this preparation of F-MuLV during previously reported studies (20) did not produce virus capable of infecting cultured mink lung cells within 2 to 3 weeks of inoculation (data not shown). Helper virus encoding amphotropic Env in an ecotropic M-MuLV background (AM-MuLV) was produced by transfection with pAM (19).

Stocks of pseudotyped SFFV_{AP-L} (26) were made by exposing the SFFV nonproducer cell line Tf-2 (a gift from S. Ruscetti) to F-MuLV or AM-MuLV, passaging the exposed cells for approximately 2 weeks, harvesting culture supernatant 24 h after changing the medium, and freezing at -70°C . The susceptibility of mice to SFFV-induced focus formation was scored 9 days after intravenous inoculation via the

FIG. 1. Increased susceptibility of F-MuLV-inoculated mice to the spleen focus-forming activity of SFFV(AM-MuLV). BALB/cByJ mice were inoculated with culture medium or F-MuLV and were challenged with SFFV(AM-MuLV) 9 days later. Nine days after challenge, the spleens were removed and fixed in Bouin's solution and macroscopic foci were scored. Spleens shown at left are from BALB/cByJ mice inoculated with culture medium and then challenged with approximately 30 focus-forming units of SFFV(AM-MuLV). Spleens shown at center are from mice inoculated with 10^6 XC PFU of F-MuLV and then with the same dose of SFFV(AM-MuLV). Spleens shown at right are from BALB/cByJ mice inoculated with 10^6 XC PFU of F-MuLV and then 1/10 the dose of SFFV(AM-MuLV). Mice inoculated with culture medium or F-MuLV alone do not develop splenic foci (data not shown).

intraorbital sinus by fixing spleens in Bouin's solution (Sigma) and counting macroscopic foci (2).

Susceptibility of viremic mice to challenge with SFFV. To measure F-MuLV-induced changes in susceptibility to superinfection, 12 female BALB/cByJ mice, 11.6 weeks old, were inoculated intravenously with 10^6 XC PFU of F-MuLV in 0.2 ml of culture medium per mouse. Fourteen control animals were inoculated with the same volume of culture medium alone. Nine days later, four of the F-MuLV-inoculated and eight of the mock-inoculated mice were challenged with undiluted or serially diluted doses of SFFV(F-MuLV). The spleen focus-forming activity of SFFV(F-MuLV), scored with F-MuLV-preinfected mice, was 1.5% that scored with control mice (data not shown), indicating that retroviral interference of the same order of magnitude as that reported previously had been established (20).

Of this group of mice, six mock-inoculated and eight F-MuLV-inoculated subjects were challenged with SFFV(AM-MuLV) to test susceptibility to superinfection independently of retroviral interference. A diluted stock of SFFV(AM-MuLV) used to inoculate control mice produced an average of 39 spleen foci per 0.2 ml (spleens shown at left of Fig. 1). The same inoculum given to F-MuLV-preinfected mice produced splenomegaly and many more spleen foci in four of four subjects tested (spleens shown at center of Fig. 1). Four F-MuLV-preinfected mice challenged with 1/10 this dilution of SFFV(AM-MuLV) produced ~ 140 foci per 0.2 ml (spleens shown at right of Fig. 1), indicating that susceptibility to the spleen focus-forming activity of SFFV(AM-MuLV) was increased by a factor of about 30 in adult mice 9 days after inoculation with F-MuLV. Similar results were obtained from four other experiments (data not shown).

This increase cannot be explained by rescue of SFFV viral genomes from nonerythroid cells by F-MuLV already present in a viremic mouse. Although rescue of SFFV by F-MuLV presumably occurs, only 1% of the rescued virions are likely to produce a spleen focus because F-MuLV-infected mice are 1% as susceptible to SFFV(F-MuLV) as are control mice (20). To increase the spleen focus-forming activity of SFFV by a factor of 30 in this context, 3×10^3 SFFV(F-MuLV) viral particles would have to be produced for each SFFV(AM-MuLV) viral particle inoculated. At least 6 days are required for the formation of detectable

spleen foci after inoculation of SFFV (30a); thus, the number of foci observed after inoculation reflects infection events that occur within the first 3 days of a 9-day assay. The viral spread that could occur during this time is enough to account for less than 2% of the spleen foci observed in the experiments described here. [The number of infected spleen cells doubles after approximately 14 h, assuming that the rate of spread of SFFV(F-MuLV) is no greater than that of F-MuLV itself and given that the number of infected cells increases by a factor of 10^4 from 1 to 9 days after inoculation of F-MuLV (20).] Therefore, nearly all spleen focus formation must be caused directly by the amphotropic SFFV inoculum, meaning that F-MuLV increases susceptibility to SFFV by affecting target cells before superinfection.

F-MuLV-induced hematopoiesis. Inoculation with F-MuLV may enhance susceptibility to the spleen focus-forming activity of SFFV by inducing an increase in the number of erythroid targets available for transformation. This hypothesis was tested by inoculating mice with F-MuLV or with culture medium alone and then measuring the colony-forming activities of committed progenitor cells present in the spleen.

To enumerate splenic progenitors, spleens were removed, perfused in Iscove's modified Dulbecco's medium containing 10% fetal bovine serum (Hyclone), and scraped through a wire mesh. The samples were counted to score nucleated splenocytes, pelleted, treated for 5 to 10 min with erythrocyte lysis buffer (Sigma), pelleted again, and washed twice in Iscove's modified Dulbecco's medium containing 10% serum before being counted in the presence of trypan blue. Some cells were serially diluted, exposed to 2,000 rads in a ^{137}Cs irradiator, and seeded as infectious centers in the UV-XC plaque-forming assay. The remaining cells were resuspended to 4×10^6 cells per ml (control samples) or 10^6 cells per ml (F-MuLV-inoculated samples) and were counted again to confirm cell densities.

Duplicate cultures were established from the spleen of each mouse tested by seeding 2×10^5 mock-inoculated or 5×10^4 F-MuLV-inoculated splenocytes per ml in Iscove's modified Dulbecco's medium containing 0.9% methylcellulose, 1% deionized bovine serum albumin, 30% fetal bovine serum, 10% pokeweed mitogen-conditioned spleen cell medium, and 1 U of human recombinant erythropoietin (a gift from B. Baranski). The addition of conditioned medium obtained from pokeweed mitogen-stimulated spleen cells, cultured for 7 days at 2×10^6 cells per ml, potentiates the erythropoiesis induced by erythropoietin and supports the clonal proliferation of granulocytic and monocytic progenitors (18). Although more control than infected splenocytes were generally used, the number of colonies produced in each case showed a linear response to the number of splenocytes seeded for a portion of the samples tested (data not shown).

Mice were tested for increased hematopoiesis 4 days after inoculation of 9.6-week-old mice, 9 days after inoculation of 6-week-old mice, and 9 days after inoculation of 11.6- and 12-week-old mice. Only 11.6- and 12-week-old mice showed detectable increases 9 days after inoculation of F-MuLV.

Splenic hematopoiesis 4 days after exposure to F-MuLV. Because retroviral interference to SFFV challenge was previously shown to be established as soon as 4 days after inoculation of F-MuLV (20), 9.6-week-old mice inoculated with medium ($n = 4$) or 10^6 XC PFU of F-MuLV ($n = 8$) were assayed for splenic progenitor activity after 4 days. Mice inoculated in parallel and then challenged with SFFV(F-MuLV) superinfection were roughly 8% as suscep-

TABLE 1. Splenic hematopoiesis after inoculation with F-MuLV^a

Inoculum ^b	Hematocrit	No. of infectious centers/ 10 ⁵ splenocytes	Cells/spleen	CFC/10 ⁵ cells ^c	CFC/spleen
M1	50	0 ^d	1.7×10^8	17	2.8×10^4
M2	52	0	1.7×10^8	19	3.1×10^4
M3	49	0	1.8×10^8	10	1.7×10^4
M4	50	0	1.5×10^8	20	3.0×10^4
Mean ^e	50 ± 1	0	$1.7 \times 10^8 \pm 0.1 \times 10^8$	16 ± 4	$2.7 \times 10^4 \pm 0.6 \times 10^4$
F1	50	2.3×10^3	2.1×10^8	31	6.4×10^4
F2	50	4.8×10^3	2.3×10^8	44	10×10^4
F3	48	5.3×10^3	2.3×10^8	49	11×10^4
F4	49	1.5×10^3	2.2×10^8	77	17×10^4
F5	49	5.6×10^3	2.0×10^8	33	6.6×10^4
F6	48	5.5×10^3	2.2×10^8	55	12×10^4
F7	40	4.3×10^3	2.5×10^8	43	11×10^4
F8	49	6.1×10^3	2.1×10^8	62	13×10^4
Mean ^e	48 ± 3	$4.4 \times 10^3 \pm 1.7 \times 10^3$	$2.2 \times 10^8 \pm 0.2 \times 10^8$	49 ± 15^f	$1.1 \times 10^5 \pm 3.4 \times 10^4$

^a Female BALB/cByJ mice, 11.6 weeks old, were tested 9 days after inoculation.

^b Mice were inoculated with culture medium (animals M1 to M4) or with 10^6 PFU of F-MuLV (animals F1 to F8).

^c Values shown are the averages from duplicate assays.

^d Mock-inoculated mice produced <1 infectious center per 10^5 splenocytes.

^e Values shown are the means $\pm$ standard deviations.

^f Statistical significance of increased hematopoiesis in infected mice was $P = 0.003$ by the Wilcoxon rank sum test.

tible as mock-infected controls (data not shown). This interference indicated that the majority of erythroid progenitor cells in these mice that proliferate upon infection by SFFV were already infected by F-MuLV 4 days after inoculation. Despite this finding, the hematopoietic activity of infected animals did not show an increase relative to that of mice free of virus at the time of superinfection challenge (data not shown).

Splenic hematopoiesis in 6-week-old mice. The initial observation that F-MuLV infection increased susceptibility to SFFV(AM-MuLV) was made with mice that were 4 to 6 weeks old at the time of inoculation (20). To determine whether hematopoiesis was induced in mice 6 weeks of age, eight BALB/cByJ mice were inoculated with 2×10^5 XC PFU of F-MuLV and were compared with mock-inoculated controls ($n = 4$). Clonal culture of explanted splenocytes 9 days after inoculation did not reveal increased hematopoietic activity in these F-MuLV-infected animals (data not shown).

Splenic hematopoiesis in adult mice. To determine whether virally induced hematopoiesis was detectable in adult mice, four 11.6-week-old BALB/cByJ mice were inoculated with medium alone and eight were inoculated with 10^6 XC PFU of F-MuLV. Nine days after inoculation, splenocytes were clonally cultured, and colonies were scored 7 days later (Table 1). The spleens from mock-inoculated mice contained an average of 2.7×10^4 colony-forming cells (CFC), but the number of CFC in the spleens of F-MuLV-inoculated mice was increased by a factor of 4, to 1.1×10^5 . Calculation by a one-sided Wilcoxon rank sum test of the statistical significance of the difference in these measurements showed that $P = 0.003$.

This analysis demonstrated that inoculation of adult mice with F-MuLV increased the number of hematopoietic progenitor cells by a factor of 4 at the same time that susceptibility to SFFV(AM-MuLV) was increased 30-fold. The effect was reproducible. In a separate experiment, 12-week-old BALB/cByJ mice tested 9 days after inoculation of 2×10^5 XC PFU of F-MuLV showed hematopoietic progenitor cell activity that was increased by a factor of 3 ($P = 0.003$) and

susceptibility to SFFV(AM-MuLV) that was increased by a factor of 20 ($P = 0.005$; data not shown). The amount of virus used was the same as that used to inoculate 6-week-old mice, which did not show increased hematopoiesis, indicating that the age of the animals is a determinant of detectable increases in hematopoiesis.

Determination of erythrocyte volume. Inoculation of newborn IRW mice with F-MuLV has been shown to induce hemolytic anemia followed by a presumably compensatory elevation in reticulocyte counts within 2 to 3 weeks (28). To determine whether induction of an anemic state in adult BALB/c mice is the cause of increased hematopoiesis in the present study, the volumes of circulating erythrocytes in the 11.6-week-old mice that were tested for splenic hematopoiesis were measured. Nine days after inoculation of 10^6 XC PFU of F-MuLV, the mice were bled from the intraorbital sinus into a heparinized capillary tube which was centrifuged and scored for erythrocyte volume as a percentage of total blood volume (hematocrit; Table 1). The mean hematocrit values determined for the infected and uninfected sets of animals were statistically identical, indicating that F-MuLV-infected mice were not detectably anemic at the same time that increased hematopoietic activity was observed. The induction of a hemolytic anemia, therefore, is not an obvious cause of increased hematopoiesis in these F-MuLV-infected mice. However, it is possible that any reduction in hematocrit values is rapidly recovered in adult mice (see below).

Identification of hematopoietic lineages grown in clonal culture. Inoculation of mice with F-MuLV reproducibly increased susceptibility to SFFV(AM-MuLV) by a factor of up to 30 but increased the number of CFC detectable in the clonal culture system by no more than a factor of 4. This outcome could be explained if erythropoiesis alone were affected such that three of every four colonies cultured from infected mice were erythroid. To determine whether F-MuLV-induced hematopoiesis was restricted to erythroid progenitor cells, cells from the colonies listed in Table 1 and from the mice inoculated with 2×10^5 XC PFU of F-MuLV 12 weeks after birth (see above) were examined morpholog-

TABLE 2. Morphology of colonies cultured from F-MuLV-infected splenocytes

Splenocyte culture source ^a	% showing the following colony types ^b :						Total tested
	G	M	GM	E	MEG	GEMM	
Expt 1							
Mock inoculated	20	52	14	11	2	0	44
F-MuLV inoculated	16	53	19	13	0	1	70
Virus production positive ^c	1/3	9/15	3/6	8/9	NA ^d	0/1	34
Expt 2							
Mock inoculated	32	37	20	10	2	0	41
F-MuLV inoculated	49	27	14	10	0	0	66
Virus production positive ^c	11/25	4/9	4/6	3/3	NA	NA	43

^a Splenocytes explanted from mock-inoculated or F-MuLV-inoculated mice were cultured for colony formation. Samples shown under experiment 1 were obtained from the cultures described in Table 1. Samples shown under experiment 2 were obtained from colonies cultured from 12-week-old mice.

^b G, granulocytic; M, monocytic; GM, granulocytic-monocytic; E, erythroid; MEG, megakaryocytic; GEMM, mixed.

^c Proportion of colonies which scored positive when tested as infectious centers in the UV-XC plaque assay. Colonies tested for virus production were a subset of those tested for colony type.

^d NA, not applicable.

ically to identify the colony type and were tested for virus production. Seven days after establishment of the cultures, when CFC were scored, colonies were removed randomly with a drawn-out Pasteur pipette, centrifuged in microtiter plate wells, and rinsed with phosphate-buffered saline. In one of the two experiments, sheep erythrocytes were added prior to centrifugation to improve recovery of the cells. One-half of each sample was exposed to 2,000 rads in a ¹³⁷Cs irradiator and plated over NIH 3T3 cells for analysis in the UV-XC infectious center assay. The remaining cells were cytocentrifuged onto microscope slides, fixed in methanol, and stained in Wright-Giemsa solution (Sigma) with an automated Hematek slide-staining machine.

Microscopic examination of the stained cells from cultures initiated with either control or F-MuLV-infected splenocytes showed similar distributions of colony types (Table 2, experiments 1 and 2). Comparison of control and virus-treated populations within each experimental set showed that granulocytic, monocytic, granulocytic-monocytic, and erythroid colonies were approximately equally represented. This result indicated that the increased hematopoiesis observed after inoculation of F-MuLV was not restricted to the erythroid lineage.

Ecotropic virus was present in each of these lineages (Table 2). The overall frequencies of virus production from the colonies analyzed in these two experiments were 64 and 51%, respectively. Colonies cultured from the splenocytes of mock-inoculated mice were not positive for virus production when tested (data not shown). If it is assumed that virus production by any of the products of clonal expansion from a progenitor cell means that the progenitor itself was infected, this analysis showed that at least half of the splenic progenitors present in mice 9 days after inoculation with F-MuLV and capable of growth in methylcellulose had been infected.

The significance of F-MuLV-induced hematopoiesis. F-MuLV was hypothesized to induce hematopoiesis because viremic mice became more susceptible to the spleen focus-forming activity of SFFV(AM-MuLV) but not to that of SFFV(F-MuLV). Enumeration of splenic CFC showed an increase by a factor of 4 after inoculation of adult mice with F-MuLV, at the same time that susceptibility to SFFV(AM-MuLV) was increased by a factor of 30. Morphological analysis of the colonies cultured from splenic progenitors

showed involvement of the myeloid and erythroid lineages, with evidence of virus production in a majority of the colonies tested. Therefore, F-MuLV-induced susceptibility to SFFV(AM-MuLV) was correlated with a generalized and rapid increase in hematopoiesis.

Ecotropic MuLV-induced malignant transformation is the result of a complex series of events, involving at the least generation of a polytropic mink cell focus-forming virus (MCF; 9) by recombination of the inoculated MuLV genome with those of endogenous retroviruses and insertional activation of cellular proto-oncogenes (10, 21). Increased hematopoiesis could accelerate leukemogenesis by contributing to the frequency of these events (as described in reference 16). MuLV-induced hematopoiesis has been reported elsewhere (4, 17, 31), but the mice were inoculated as newborns and showed increases only after 4 to 6 weeks. Clonal culture experiments with adult mice may permit earlier detection of virally induced changes in the number of hematopoietic progenitor cells. This may be true because the proportion of dividing splenocytes decreases in mice from 2 to at least 8 weeks after birth (31), thereby providing a lower background of progenitor cell production to which virus-induced production was normalized. The absolute numbers of progenitor cells enumerated in the present study fell by a factor of about 2 from 6 to 12 weeks of age (data not shown), a reduction sufficient to abolish the statistical significance of increased hematopoiesis measured by the clonal culture assay. Increased susceptibility to superinfection in F-MuLV-infected mice did not appear to vary as a function of age. The spleen focus-forming assay used to determine susceptibility to superinfection may permit a more sensitive measurement of hematopoietic activity, but with much less precision than clonal culture experiments, because susceptibility to infection by MuLVs is dependent both on the number and on the cycling status of target cells.

The rapid consequences of inoculation of F-MuLV indicate that ecotropic F-MuLV, and not its polytropic derivatives, directly induces increased hematopoiesis. Polytropic viruses become detectable in the 3rd week following inoculation of newborn mice with F-MuLV (3, 32). It is not likely that polytropic viruses are generated more quickly in adult mice than in newborn mice, because adult mice develop erythroleukemia after a much longer latency than newborns (3), and adult BALB/c mice tested previously with the same

viral stocks used in the present study showed no production of viruses with mink cell tropism 18 days after inoculation of F-MuLV (20 and data not shown). Therefore, F-MuLV alone is the probable agent of the increased hematopoiesis shown here.

The hematopoietic activity of F-MuLV was consistent either with a rapid response to hemolytic anemia or with a mitogenic effect mediated by viral gene expression. F-MuLV-induced anemia has been reported only after the inoculation of newborn mice, possibly because erythrocyte blood volume was slowly recovered in animals also pressed to expand their overall blood volumes (27, 28). The hematocrit values reported here (Table 1) showed that anemia was not correlated with increased hematopoiesis, but adult mice may be capable of rapidly compensating for losses due to hemolysis. Indeed, the dependence on age of F-MuLV-induced hematopoiesis with the mice tested here may in part reflect an increased capacity of mice with matured hematopoietic organs to compensate rapidly for small deficits in erythrocyte volume. If the mechanism inducing hemolytic anemia was effective within 9 days after inoculation of virus, the compensating hematopoietic response must include multiple lineages, because granulocytic, monocytic, granulocytic-monocytic, and erythroid stem cells were represented with equivalent frequencies in the spleens of viremic mice relative to those of nonviremic controls.

Alternatively, F-MuLV infection may induce a mitogenic response in each of these lineages through viral gene expression. Analysis of virus production showed the presence of ecotropic virus in at least half of the splenic CFC tested, consisting of both myeloid and erythroid progenitors. A similar range of expression was reported for retroviral vector constructs containing the enhancer/promoter sequences of the F-MuLV proviral long terminal repeat (11). F-MuLV gene products may mitogenically stimulate the cells that the virus infects or perhaps accessory cells secreting growth factors. The gp55 membrane glycoprotein of SFFV is the best-characterized retroviral gene product having mitogenic effects (15). The envelope glycoprotein of polytropic viruses has also been reported elsewhere to stimulate hematopoietic cell growth (14, 33), and ecotropic Moloney-MuLV has been reported to immortalize mature B cells efficiently in vitro (25).

Whichever model is accurate, the demonstration that F-MuLV induces increased hematopoietic activity soon after infection provides a useful explanation for the erythro-leukemogenic synergism between F-MuLV and its polytropic derivative, F-MCF (3). Although expression of polytropic gene products is mitogenic in some cell lines (14, 33), most F-MCF isolates are not by themselves leukemogenic (7). F-MuLV-induced expansion of the hematopoietic cell population may provide more targets for infection and proviral integration by F-MCF, making more likely the additional leukemogenic events necessary for malignant transformation.

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