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Leukemia Induction by a New Strain of Friend Mink Cell Focus-Inducing Virus: Synergistic Effect of Friend Ecotropic Murine Leukemia Virus

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A new strain of Friend recombinant mink cell focus-inducing retrovirus, FMCF-1-E, was found to induce leukemias in NFS and IRW mice. Although the isolate was obtained from a stock of FMCF-1 (Troxler et al., J. Exp. Med. 148:639–653, 1978), FMCF-1-E was distinguishable from FMCF-1 by oligonucleotide fingerprinting and antigenic analysis, using monoclonal antibodies. These analyses suggested that FMCF-1-E is a distinct FMCF isolate rather than a simple variant of FMCF-1. After neonatal inoculation, the latency for leukemia induction was 3 to 8 months. A similar long latency was also seen when Friend murine leukemia virus 57 was inoculated into adult (6-week-old) IRW mice. However, sequential inoculation of FMCF-1-E at birth followed by Friend murine leukemia 57 at 6 weeks of age led to a shortened latency period (2.5 to 4 months). Only neonatal inoculation of Friend murine leukemia virus 57 was able to induce a more rapid appearance of leukemia. The leukemia cell type in the majority of cases, regardless of virus inoculation protocol, was erythroid, but occasional myeloid, lymphoid, and mixed leukemias were also observed. In contrast to NFS and IRW mice, BALB/c mice were resistant to leukemia induction by FMCF-1-E and also showed some transient resistance to leukemia induction by Friend murine leukemia virus 57.

Many retroviruses can induce neoplastic transformation in mice (23). Although *onc* genes responsible for transformation have been identified in several rapidly transforming viruses, in slowly transforming viruses such genes have been difficult to identify (2, 20). This problem has become more complex due to the discovery that after inoculation or endogenous expression of ecotropic murine leukemia viruses (MuLV) new dual-tropic mink cell focus-inducing (MCF) viruses are generated (18, 19, 25). These viruses appear to be recombinants between ecotropic virus and endogenous non-ecotropic viral gene sequences present in normal cellular DNA (4, 5, 10, 14, 17, 22, 30, 39, 44). MCF viruses may be important in some types of leukemogenesis in mice because their appearance often correlates well with the onset of leukemia (25). It has been postulated that certain MCF viruses may be directly responsible for the neoplastic transformation induced by inoculation or expression of ecotropic viruses such as Friend, Moloney, Rauscher, and AKR viruses (18, 25, 48). In support of this possibility, several MCF viruses derived from Moloney or Rauscher MuLV were capable of inducing leukemia in the absence of ecotropic virus (18, 19, 21, 50, 51). In addition, certain MCF viruses were found to accelerate the appearance of leukemia in AKR mice (11, 32); however, this acceleration appeared to be dependent on the expression of ecotropic AKR virus (12).

In the Friend helper virus (F-MuLV) system, MCF viruses have frequently been isolated from leukemic tissues (6, 16, 27, 49); however, MCF virus expression does not appear to be required for F-MuLV-induced leukemogenesis in some hemapoietic cell types of certain mouse strains (7). The role of F-MuLV-induced MCF (FMCF) viruses in leukemogenesis is further confused by the observation that many FMCF isolates do not induce leukemia when inoculated in the absence of other viruses (16, 41). Several FMCF viruses have been reported to be oncogenic (27); however, mink cells infected with these viruses gave XC-positive foci, which suggested that ecotropic F-MuLV still contaminated these preparations. Because of these conflicting reports, we decided to screen several FMCF strains for the ability to induce leukemia in the absence of F-MuLV. One virus was found to be oncogenic in the absence of F-MuLV but appeared reduced in virulence compared with F-MuLV. However, a synergistic effect on leukemia induction could be demonstrated by sequential inoculation of FMCF virus at birth followed by F-MuLV at 6 weeks of age.

MATERIALS AND METHODS

Mice. NFS, inbred NIH Swiss mice were obtained from the National Institutes of Health, Bethesda, Md. IRW mice were derived at the Rocky Mountain Laboratory (RML) by the inbreeding of an outbred colony of Carworth Farms White mice (7). BALB/c mice were the nu/+ and +/+ littermates of BALB/c-nu/nu mice obtained from Harlan, Sprague-Dawley. All mice used for neonatal inoculation were bred at the RML.

Viruses and cells. F-MuLV 57 (35, 47), F-MuLV 67, FMCF 1511 and FMCF 1512 (6), and FMCF-1, referred to in this paper as FMCF-1-T (49), were propagated on SC-1 (24), Dunii (5), or mink CCL64 (26) cells. FMCF-1-T was obtained from infected mink cells received at RML from D. Troxler in 1979; the supernatant fluid was removed and stored at −70°C before any passages of these cells at RML. A new stock of FMCF-1-T was made by infection of Dunii cells with this original supernatant fluid in 1983, and these cells and the virus they produced were analyzed in the present report. The origin of FMCF-1-E was as follows: FMCF-1-infected Fisher rat embryo cells were obtained from D. Troxler by L. Evans in 1978. Virus from these cells was previously analyzed by oligonucleotide fingerprinting (14). These cells were maintained in passage and were brought to RML by L. Evans in 1980. At RML, cell-free serial passages at limiting

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dilutions were made twice on mink cells and twice on SC-1 cells. Stocks from the second mink cell passage and second SC-1 passage were used to inoculate mice. The latter was designated strain FMCF-1-E. Dunii cells were infected at a 10^{-2} dilution with this virus to obtain virus for oligonucleotide fingerprinting and infected cells for antigenic analysis.

Leukemogenesis experiments. Mice less than 24 h old were inoculated intraperitoneally with 0.05 ml of undiluted tissue culture supernatant fluid containing F-MuLV 57 (500 to 5,000 XC PFU) or FMCF viruses (500 to 5,000 fluorescent focus-forming units). Mice were followed for development of splenomegaly or lymphadenopathy by weekly palpation while under ether anesthesia. Obviously enlarged spleens detectable by palpation were found to weigh 0.4 to 2.5 g (9), and mice with such splenic enlargement for more than 2 weeks were considered to be leukemic. In many cases leukemic mice were sacrificed, and cells from enlarged spleens or lymph nodes were analyzed for cell type by cytochemical staining and for virus expression by infectious center titration (7).

Titration of cell-free virus and infectious centers. Ecotropic F-MuLV was titrated by XC plaque assay on SC-1 cells (40). FMCF viruses were titrated on mink lung cells by reading the cytopathic effect on days 5 to 7 and by analysis of foci of infected cells attached to cover slips which were acetone fixed and stained with fluorescein isothiocyanate-conjugated goat anti-Moloney MuLV serum (24). To obtain more rapid and accurate titers of MCF viruses, a new monoclonal antibody-mediated fluorescent focus assay on live cell monolayers was devised, based on a modification of an autoradiographic focus assay (15). Dilutions of spleen cells were seeded onto 60-mm petri dishes containing 1×10^5 SC-1 or 5×10^4 Dunii cells. Four to six days later, unfixed monolayers were incubated for 0.5 h at 37°C with monoclonal antibody 514 or 48, specific for MCF viruses or F-MuLV, respectively (6, 8). Monolayers were washed twice and then incubated for 0.5 h with a 1:200 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin serum. After two additional washes, the fluid was aspirated, and dishes were inverted and examined for fluorescent foci in a Leitz Orthoplan microscope at ×80 magnification. Ten to twenty percent of the dish surface was examined, and titers were expressed as focus-forming units per 10^6 cells seeded or per ml of cell-free virus added. With the appropriate monoclonal antibodies, individual viruses in complex mixtures could be easily titrated, and with the appropriate choice of target cells, pseudotyped viruses could be detected efficiently (M. Sitbon and B. Chesebro, manuscript in preparation).

Oligonucleotide fingerprinting. Analysis of T_1 oligonucleotides was carried out as described previously (16).

Antigenic analysis. Virus-induced cell surface antigens were analyzed by using monoclonal antibodies as previously described (6, 8).

RESULTS

Leukemia induction by Friend MCF viruses. Leukemogenicity of three different FMCF virus isolates was tested by inoculation of newborn mice with virus stocks made from supernatant fluid of infected cells. In early experiments in which supernatants from mink cells were used, FMCF 1511 did not induce leukemia, whereas FMCF 1512 and FMCF-1-E did (Table 1). Because of concern that perhaps these leukemias might actually be induced by residual ecotropic F-MuLV contaminating the MCF virus stocks, attempts were made to detect F-MuLV in some mice inoculated with MCF virus. All mice inoculated with FMCF 1512 had large amounts of F-MuLV in their enlarged spleens. However, none of 14 mice inoculated with FMCF-1-E were positive for F-MuLV. Thus, it appeared that the mink cell stock of FMCF 1512 contained F-MuLV which was detectable by inoculation of baby mice but not by in vitro infection and amplification on SC-1 cells. We used this fact to eliminate the ecotropic virus by making new MCF stocks after limiting dilution cloning on SC-1 cells. In mink cells, ecotropic viruses do not spread well alone but could remain present in small amounts as phenotypic mixtures with MCF virus. In contrast, on SC-1 cells any ecotropic virus which successfully infected a cell should spread rapidly and be easily detectable. Our new MCF virus stocks made from SC-1 cells had no detectable ecotropic F-MuLV by in vitro XC testing. When these were inoculated into newborn mice, only the FMCF-1-E virus induced leukemia and none of the leukemic mice tested had evidence for reexpression of F-MuLV (Table 1).

Relationship between FMCF-1-E and FMCF-1-T viruses. We were surprised by the leukemogenicity of FMCF-1-E because we originally believed it to be identical to the FMCF-1 isolated by Troxler et al. (FMCF-1-T), which was reported not to be leukemogenic by itself in newborn NIH Swiss mice (41, 49). After observing leukemia induction by our FMCF-1-E stock, we compared this virus to FMCF-1-T virus frozen directly from a flask of infected cells obtained in 1979 from D. Troxler. These two viruses were different and could be distinguished both by antigenic analysis, using monoclonal anti-gp70 antibodies (Table 2), and by oligonucleotide fingerprinting (Fig. 1). This latter analysis indicated that FMCF-1-T and FMCF-1-E shared 30 oligonucleotides, 24 of which were also found in F-MuLV 57. However, 15 oligonucleotides present in FMCF-1-T were missing in FMCF-1-E, and these were located at many sites scattered throughout the genome. Furthermore, FMCF-1-E exhibited 13 oligonucleotides which were missing in FMCF-1-T. Thus, these viruses were not closely related by a point mutation or recombinational event. FMCF-1-T was previously shown to be a recombinant virus probably derived from F-MuLV 57 (14). In contrast, FMCF-1-E did not appear to be derived from F-MuLV 57 based on analysis of gp70 antigens. Instead, FMCF-1-E appeared to be related to some other F-MuLV strain because it expressed three envelope antigens detected by monoclonal antibodies 350, 417, and 403, which were previously found only in F-MuLV and FMCF strains unrelated to F-MuLV 57 (6). This interpretation was also

TABLE 1. Leukemia induction and F-MuLV reisolation by Friend MCF virus isolates[a]

Virus	Leukemia induction in cell stock[b]:		F-MuLV reisolation in cell stock[c]:	
	Mink	SC-1	Mink	SC-1
FMCF 1511	0/24	NT	NT	NT
FMCF 1512	6/34	0/12	5/5	NT
FMCF-1-E	22/60	92/107[d]	0/14	0/20

[a] Virus stocks were made from supernatant fluid from infected mink lung cells (CCL64) or mouse SC-1 cells. NT, Not tested.

[b] Number of mice with leukemia/total number inoculated.

[c] 10^6 cells from enlarged spleens were cocultivated with SC-1 cells. Primary and secondary passages of SC-1 cells were then analyzed for XC-positive ecotropic virus. Identity of ecotropic virus was confirmed by reactivity with F-MuLV-specific monoclonal antibody 48. Values shown are number of mice in which F-MuLV was reisolated/total number of mice tested.

[d] Includes both NFS (14/14) and IRW (78/93) mice.

supported by the finding of two oligonucleotides in FMCF-1-E which appeared to be present in F-MuLV strains other than F-MuLV 57 (unpublished data).

Kinetics of leukemia induction by FMCF-1-E in various mouse strains. The kinetics of leukemia induction by FMCF-1-E and ecotropic F-MuLV 57 were compared in three mouse strains. In IRW mice, leukemia appeared much sooner after F-MuLV 57 inoculation than after FMCF-1-E inoculation (Fig. 2A). More than 80% of mice inoculated with F-MuLV 57 had splenomegaly after 1.5 months, whereas an 80% incidence of leukemia was not observed until 6.5 months after FMCF-1-E inoculation. Similar results were observed when smaller numbers of NFS mice were used (data not shown).

BALB/c mice appeared resistant to leukemia induction by FMCF-1-E (Fig. 2B). During the 8 months shown in Fig. 2B, no leukemias were seen in FMCF-1-E-inoculated mice. After F-MuLV 57 inoculation, a group of mice developed splenomegaly at 1.5 to 2 months, but at 2 months about half of the mice had a spontaneous recovery from splenomegaly. The actual incidence of recovery was higher than the decrease from 54 to 34% shown in Fig. 2B because the 34% value included some new mice which had no previous splenomegaly. The recovery observed in individual mice was transient as most mice developed recurrent splenomegaly and died in the following months.

Since F-MuLV 57 induced more rapid leukemia than did FMCF-1-E in all three mouse strains, this data did not appear to support the idea that F-MuLV 57 induced leukemia by first generating a leukemogenic FMCF virus similar to FMCF-1-E, which then in turn independently infected and transformed the appropriate cells.

Acceleration of leukemia onset by sequential inoculation with FMCF-1-E and F-MuLV 57. Previous experiments by others showed that MCF virus-induced acceleration of leukemogenesis was dependent on expression of Akv ecotropic viruses in AKR mice (12). In the Friend virus system, a similar interaction between F-MuLV and FMCF virus would not appear to be required since FMCF-1-E induced leukemia in the absence of F-MuLV. However, in view of the slow course of leukemia induction by FMCF-1-E inoculated at birth, we wondered whether subsequent inoculation of F-MuLV would accelerate the appearance of leukemia. Previous data indicated that leukemia induction by F-MuLV 57 was reduced when virus was inoculated when mice were 5 to 7 weeks of age (42). When we used this time of F-MuLV 57 inoculation, we found that appearance of leukemia was delayed, but more than 80% of mice had leukemia by 8 months of age (6.5 months after inoculation) (Fig. 3). When mice were inoculated at birth with FMCF-1-E and at 5 to 7 weeks with F-MuLV 57, leukemia induction was markedly accelerated when compared with that observed after inoculation of either virus alone (Fig. 3). Both viruses appeared to have leukemogenic potential; however, neither contains recognized transforming genes. Therefore, it was unclear which virus had the most direct role in the actual transformation event(s). Furthermore, the mechanism leading to the accelerated leukemogenesis was not obvious.

Cell types involved in FMCF-1-E-induced leukemia. Data from several laboratories indicated that F-MuLV inoculation induces leukemias in at least three different hemapoietic cell lineages; erythroid, myeloid, and lymphoid (31, 45–47). Furthermore, different mouse strains vary in the incidence with which different lineages are involved (7, 43). To more accurately compare the disease induced by FMCF-1-E, F-MuLV 57, and the combination of these viruses, cells from enlarged spleens or lymph nodes of mice with leukemia were analyzed cytochemically. The results indicated that in all combinations of virus and mouse strain described in this study erythroid or erythroid-plus-myeloid leukemias predominated, even though occasionally other lineage combination were involved (Table 3). Furthermore, hematocrits were found to be very low (10 to 25%) in 15 of 15 IRW mice tested after the appearance of splenomegaly after FMCF-1-E inoculation, a pattern identical to that seen after inoculation of F-MuLV 57. Thus, the same spectrum of diseases appeared to be induced by F-MuLV 57, FMCF-1-E, or the combination of both viruses in the mouse strains analyzed here.

TABLE 2. Comparison of envelope protein antigenicity of FMCF-1-E, FMCF-1-T, and two strains of F-MuLV

Monoclonal antibody[a]	Antigenicity of:			
	FMCF-1-T[b]	FMCF-1-E[b]	F-MuLV 57	F-MuLV 67
307	−	−	+	+
48	−	−	+	+
273	−	−	+	+
500	+	+	+	+
350	−	⊕[c]	−	⊕
417	−	⊕	−	⊕
403	−	⊕	−	⊕
502	+	+	−	−
514	+	+	−	−
508	+	+	−	−
513	+	+	−	−
516	+	+	−	−
522	+	+	−	−
518	−	−	−	−
18-6	−	−	−	−
19-1	−	−	−	−
603	−	−	−	−

[a] Monoclonal anti-gp70 antibodies were described previously (6, 8, 36, 37).
[b] Antigenicity was tested by using a ^{125}I-labeled protein A cell surface binding assay on virus-infected Dunii or SC-1 cells as previously described (6). Uninfected cells were nonreactive with all antibodies used.
[c] Circled positive values distinguish FMCF-1-T from FMCF-1-E.

Effect of sequential FMCF-1-E and F-MuLV 57 inoculation on spread of virus infection in vivo. One possible explanation for the acceleration of leukemia induction by sequential FMCF-1-E and F-MuLV 57 inoculation is that the phenotypic mixing of the two viruses might facilitate more rapid infection of certain cell populations by one or the other virus. We have attempted to test this possibility by titrating the number of infectious centers producing F-MuLV 57 or FMCF-1-E in spleen cell populations obtained from mice inoculated with either virus alone or the sequential combination of both viruses described above. The results indicated that there was no detectable increase in the total number of cells infected by either virus in mice which had received both viruses sequentially (Fig. 4). However, our data cannot exclude the possibility that increased infection might have occurred in certain minor cell populations with a high potential of susceptibility to transformation by one of these viruses.

DISCUSSION

The present results illustrate the difficulty of removing ecotropic F-MuLV from FMCF isolates and the importance of retesting mice for ecotropic virus after the development of leukemia after MCF virus inoculation (18). Our results suggest that isolation protocols in which viruses are passed at limiting dilution alternately on mink cells and mouse cells would most likely fail to eliminate ecotropic viruses. A small

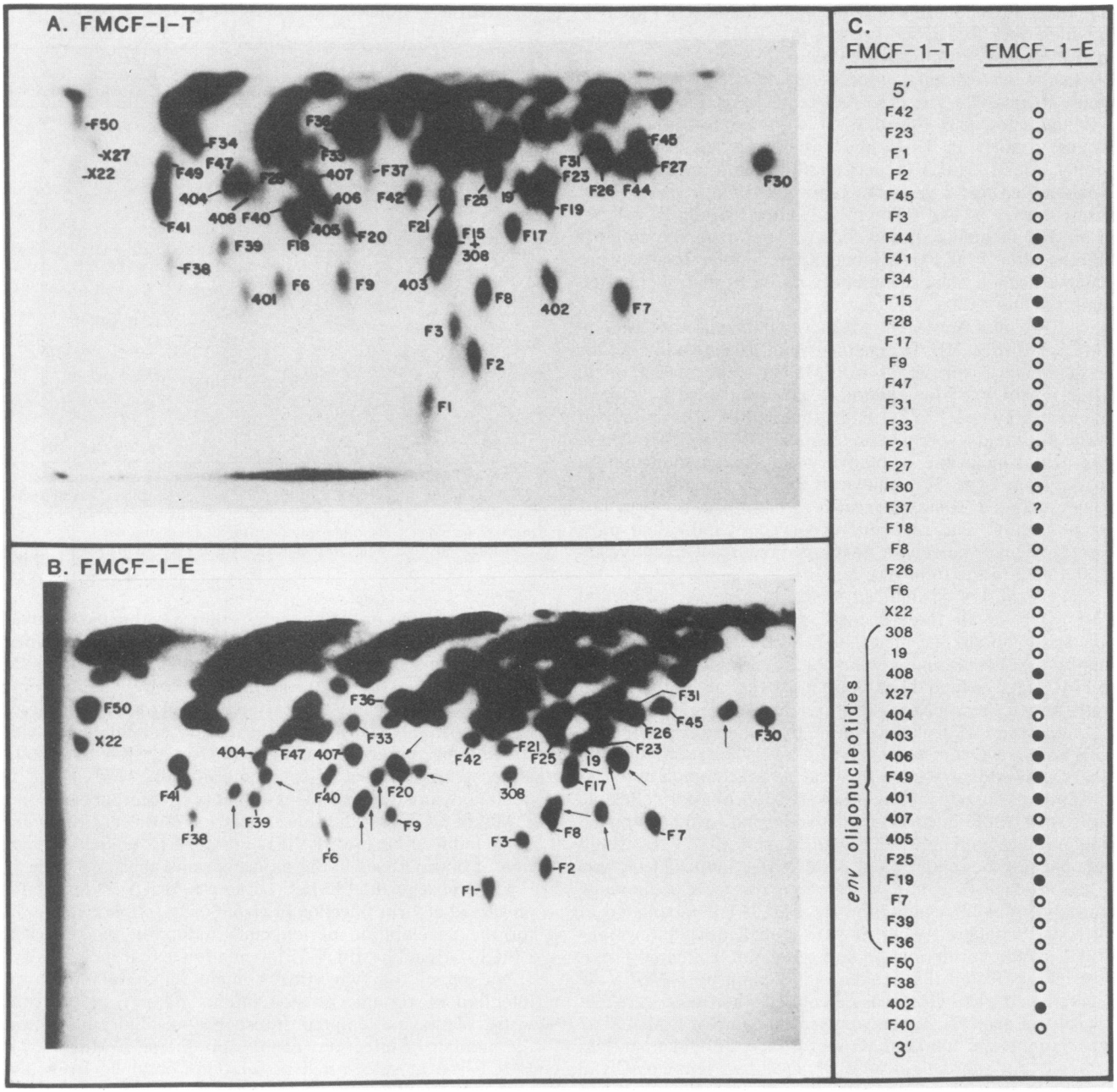

FIG. 1. RNase T_1-resistant oligonucleotides of FMCF-1-T and FMCF-1-E. FMCF-1-T and FMCF-1-E 70S RNA (~10^6 cpm) were digested with RNase T_1 and fingerprinted. Electrophoresis was from left to right, and homochromatography was from bottom to top. (A) T_1-resistant oligonucleotide fingerprint of FMCF-1-T. Oligonucleotides prefixed by an F correspond to oligonucleotides which are shared with F-MuLV 57 (14, 16). The remaining oligonucleotides which are either prefixed by an X or are designated by unprefixed numbers correspond to oligonucleotides identified in FMCF-1-T but not found in F-MuLV 57. (B) T_1-resistant oligonucleotides of FMCF-1-E. Oligonucleotides shared with FMCF-1-T are numbered identicially to those in FMCF-1-T. Oligonucleotides of FMCF-1-E which are not found in FMCF-1-T are identified by arrows. (C) T_1 oligonucleotide map of FMCF-1-T and map positions of oligonucleotides which are shared by FMCF-1-E (○) or differ from those in FMCF-1-E (●). It could not be determined whether oligonucleotide F37 was an element of FMCF-1-E; thus the map position corresponding to F37 is designated by a question mark. Oligonucleotides residing in the *env* gene of FMCF-1-T are bracketed and were deduced from nucleotide sequence data of F-MuLV 57 (29) and a FMCF (A. Ishimoto, personal communication).

fraction of phenotypically mixed ecotropic virus with an MCF envelope can infect mink cells, and, unless eliminated by two or three successive cell-free passes through mink cells, this ecotropic virus would be reexpanded to a high titer by subsequent passage on mouse cells. Such difficulties might account for the discrepancy between previous reports indicating high leukemogenicity and ability to form XC syncytia with several FMCF isolates (27) and more recent findings indicating that none of seven new FMCF isolates tested appeared to be leukemogenic (16).

We were, however, able to find one FMCF strain, FMCF-1-E, which induced leukemia in the absence of F-MuLV and

in the absence of phenotypic mixing with amphotropic MuLV (41). In this latter respect, FMCF-1-E was apparently different than FMCF-1-T. Even though the FMCF-1-E strain was originally derived from a cell culture containing FMCF-1-E, these two strains did not appear to be closely related mutants or recombinants. We are currently retesting the leukemogenicity of a recloned stock of FMCF-1-T in our NFS mice.

Previous data from many laboratories indicate that murine leukemia viruses often differ in tissue tropism of transforming effects in vivo. F-MuLV is somewhat unusual in that it appears to cause transformation in at least three different hemapoietic lineages, suggesting that one target for transformation might be a multipotential stem cell. However, in the mouse strains used in the present study, most mice showed erythroid or mixed erythroid and myeloid neoplasms. This was true not only for F-MuLV 57 but also for FMCF-1-E and for the sequential inoculation of FMCF-1-E at birth and F-MuLV 57 at 6 weeks of age. Although inoculation of these different viruses gave rise to the same spectrum of diseases, there was a marked variation in the rate of induction of leukemia. Thus, it would appear likely that FMCF-1-E and F-MuLV 57 (and other ecotropic F-MuLVs) (6) share common genes responsible for the tissue specificity of transformation and differ in gene sequences responsible for variation in rate of leukemia induction. A similar variation in rate of leukemogenesis was also observed previously when Moloney MuLV and a Moloney MCF virus were compared (51).

Several possible interpretations might explain the difference in kinetics which was seen when either F-MuLV 57 or FMCF-1-E was inoculated into newborn IRW or NFS mice (Fig. 2A). First, it has been proposed that F-MuLV indirectly induces the transformation of erythroid cells by generating FMCF viruses, which in turn transform the cells (48). It is possible that F-MuLV might generate FMCF viruses which

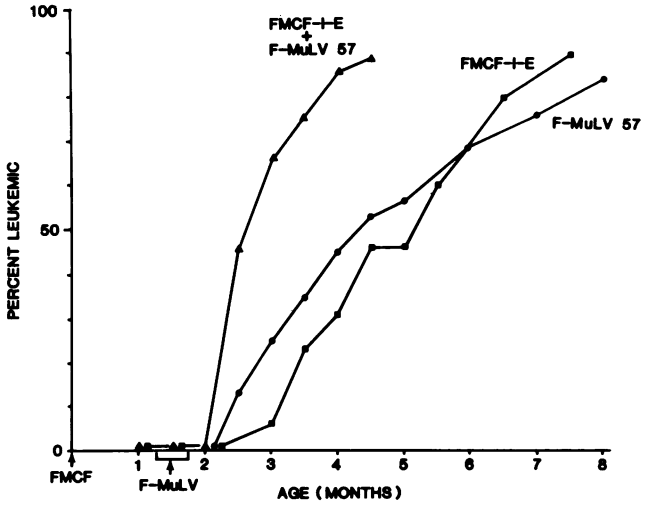

FIG. 3. Incidence of leukemia in IRW mice inoculated at birth with FMCF-1-E (■), at 5 to 7 weeks of age with F-MuLV-57 (●), or at birth with FMCF-1-E and at 5 to 7 weeks with F-MuLV-57 (▲).

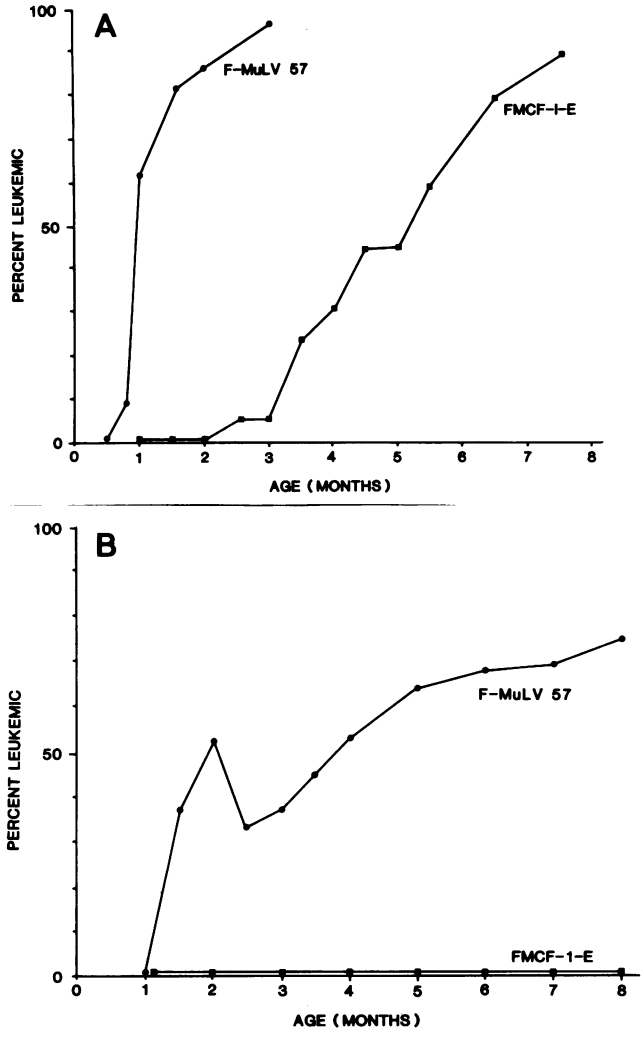

FIG. 2. Incidence of leukemia in IRW (A) or BALB/c (B) mice inoculated at birth with F-MuLV-57 (●) or FMCF-1-E (■).

TABLE 3. Predominant cell types in enlarged spleens and lymph nodes of mice inoculated with various viruses[a]

Cell types[b]	No. of mice inoculated						
	BALB/c		NFS		IRW		
	57	E	E	57	57 (adult)	E	E + 57 (adult)
Erythroid	8	1	4	16	14	28	18
Myeloid	1	1	0	3	1	1	0
Lymphoid	0	0	0	0	2	3	0
Erythroid + lymphoid	1	0	0	1	2	1	1
Erythroid + myeloid	7	1	1	8	2	7	3
Myeloid + lymphoid	0	1	0	0	0	0	0
Erythroid + myeloid + lymphoid	1	1	0	0	1	0	0

[a] 57, F-MuLV 57 inoculated into newborn BALB/c and IRW mice; E, FMCF-1-E inoculated into newborn BALB/c, NFS, and IRW mice; 57 (adult), F-MuLV 57 inoculated into 5- to 7-week-old IRW mice; E + 57 (adult), FMCF-1-E inoculated into IRW mice at birth and F-MuLV 57 inoculated at 5 to 7 weeks of age.

[b] Predominant cell types of enlarged spleens or lymph nodes were determined by staining with Sudan black and α-naphthyl butyrate esterase as previously described (7).

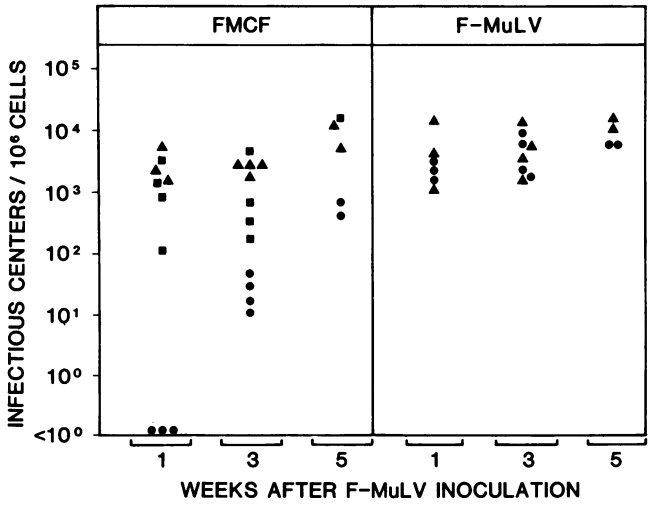

FIG. 4. Titration of infectious centers per 10^6 spleen cells on Dunii cell monolayers, using a fluorescent focus assay with monoclonal antibodies (514 for FMCF and 48 for F-MuLV). Mice were inoculated with FMCF-1-E at birth (■), with F-MuLV-57 at 6 weeks of age (●), or with FMCF-1-E at birth and F-MuLV 57 at 6 weeks of age (▲). Mice from all three groups were sacrificed for assay at 1, 3, and 5 weeks after the F-MuLV inoculations.

are more oncogenic than FMCF-1-E, thus leading to more rapid leukemogenesis than does direct inoculation of newborns with FMCF-1-E. This explanation appears to be less likely because most FMCF viruses isolated from mice inoculated with F-MuLV are not oncogenic when inoculated by themselves (16). Second, F-MuLV 57 and FMCF-1-E might differ at gene sequences responsible for the rate of transformation. Both the envelope region (33) and long terminal repeat regions (3, 13, 38) have been implicated as being important in tissue tropism of transformation. F-MuLV and FMCF-1-E differ in both of these regions, although they appear to give the same types of leukemias in the mice tested. Third, differences in envelope protein structure might allow infection of susceptible target cells more efficiently by F-MuLV 57 than by FMCF-1-E. Previous data indicate that retroviral envelope proteins play an important role in determining infectivity (1, 28, 52). Thus, a change in viral envelope could result in infection of a different population of cells in vivo. This in turn might affect the rate of leukemogenesis, depending on the susceptibility of a given cell to transformation either by the infecting virus or by a recombinant MCF virus generated after infection. Fourth, the increased yield of F-MuLV from initially infected cells might facilitate infection of marginally infectable, but easily transformed, cell populations. Fifth, the observed disease may actually consist of two stages, an early blastogenic stage and a later neoplastic stage. F-MuLV might induce the early stage, and FMCF-1-E might induce the later stage. Lack of a precise test capable of accurately distinguishing these stages makes this a difficult possibility to prove, but previous results showing regression of splenomegaly in some F-MuLV-inoculated mice after erythrocyte transfusion lend support to this concept (34).

The present results indicated the presence of an interesting interaction between F-MuLV 57 and FMCF-1-E virus to produce a more rapid leukemia when these viruses were given sequentially. Our previous results suggested that F-MuLV 57 in the absence of generation of detectable MCF virus was capable of leukemogenesis in certain hemapoietic lineages of certain mouse strains (7). The present results show that FMCF-1-E in the absence of any ecotropic MuLV could also induce transformation. Thus, each virus appeared capable of inducing transformation in the absence of other viruses. The mechanism of acceleration of leukemogenesis when both viruses were present remains unclear. The two viruses might be generating a new recombinant virus with more rapid leukemogenic activity than that of either inoculated virus. In contrast, the two input viruses might complement each other so that each virus increased the rate of a different step required in the transformation process. For example, it seems likely that the phenotypic mixing of the two viruses would facilitate the spread of one or the other virus to additional cell populations not previously infected by a particular virus. If one such subpopulation was highly susceptible to transformation, this could result in the more rapid appearance of leukemia. In fact, such a mechanism could be operative in newborn IRW or NFS mice inoculated with F-MuLV alone, as generation of new FMCF viruses is very rapid in these mice (27), and such viruses would phenotypically mix with F-MuLV in vivo. Our attempts to document an increase in the spread of F-MuLV 57 or FMCF-1-E in mice given the sequential inoculation protocol were not successful. This observation indicated that if such effects occur they probably involve minor subpopulations of cells too low in frequency to be noticeable against the high background of cells infected by either virus inoculated alone.

We were surprised to note that 5- to 7-week-old IRW mice inoculated with F-MuLV 57 developed a high incidence of leukemia. The main difference between inoculation of newborns and adults appeared to be the more gradual slope of the leukemia incidence curve in the adults. We also noted a high frequency of MCF virus expression in these mice (Fig. 4). These observations are in contrast with those made by Ruscetti et al. (42) in outbred NIH Swiss mice in which MCF expression and leukemia incidence dropped drastically when older mice were inoculated. It is unclear whether mouse strain differences or technical differences in the experimental procedures account for these discrepancies.

The present results also demonstrated a difference in susceptibility of various mouse strains to both F-MuLV 57 and FMCF-1-E. This was most striking in the case of FMCF-1-E, which induced no leukemias in BALB/c mice within 8 months. A few leukemias were noted at 13 months (data not shown), but because of the very long latency it was difficult to be sure whether these were virus-induced or spontaneous neoplasmas. The transient recovery from F-MuLV-induced leukemia seen in these BALB/c mice was unexpected. Others have reported that BALB/c mice are susceptible to this virus and did not observe evidence of recovery (41). It seems likely that our method of following mice by spleen palpation would facilitate detection of such a recovery phenomenon (9). However, it is also possible that the BALB/c sublines used by different workers have genetic differences which could account for these new findings.

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