Differential Disease Restriction of Moloney and Friend Murine Leukemia Viruses by the Mouse *Rmcf* Gene Is Governed by the Viral Long Terminal Repeat

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Summary

Neonatal CxD_2 (*Rmcf*) and Balb/c (*Rmcf*) mice inoculated with Moloney murine leukemia virus (M-MuLV) exhibited approximately equivalent time course and pathology for disease. CxD_2 mice showed only slightly reduced presence of Moloney mink cell focus-forming virus (M-MCF) provirus as seen by Southern blot analysis compared to Balb/c mice. This lack of restriction for disease and spread of MCF was in sharp contrast to that seen for CxD_2 mice inoculated with Friend murine leukemia virus (F-MuLV), where incidence of disease and propagation of MCFs were severely restricted, as previously reported. Inoculation of CxD_2 mice with FM-MuLV, a recombinant F-MuLV virus containing M-MuLV LTR sequences (U3 and R), resulted in T cell disease of time course equal to that seen in Balb/c mice; there also was little restriction for propagation of MCFs. This indicated that presence of the M-MuLV long terminal repeat (LTR) was sufficient for propagation of MCFs in CxD_2 mice. Differing restriction for F-MuLV vs. M-MuLV in CxD_2 mice was explained on the basis of different "MCF propagator cells" for the two viruses. It was suggested that cells propagating F-MCF (e.g., erythroid progenitors) are blocked by endogenous MCF-like $gp70^{env}$ protein, whereas cells propagating M-MCF (e.g., lymphoid) do not express this protein on their surface. F-MuLV disease in CxD_2 mice was greatly accelerated when neonates were inoculated with a F-MuLV/F-MCF pseudotypic mixture. However, F-MCF provirus was not detectable or only barely detectable in F-MuLV/F-MCF-induced tumors, suggesting that F-MCF acted indirectly in induction of these tumors.

Moloney murine leukemia virus (M-MuLV)[1] induces T cell lymphoma with a typical latency of 3–4 mo when inoculated into neonatal mice. One of the events observed in M-MuLV–induced disease is the appearance of mink cell focus-forming (MCF) viruses, which result from recombination of the input ecotropic virus with endogenous polytropic retroviral sequences (1, 2). In mice, MCF proviruses are found in M-MuLV–induced tumors (3–5), and are also observed at significant circulating titres at preleukemic times (6). They have been proposed to be the "proximal leukemogens" in MuLV-induced disease (7–9). We have recently reported evidence that MCFs may also play a role early in M-MuLV–induced leukemogenesis, by participating in induction of preleukemic hematopoietic hyperplasia of the spleen (5, 10). The proposed role of MCFs in this process was to induce suppression of bone marrow hematopoiesis by combined infection with M-MuLV, leading to compensatory extramedullary hematopoiesis in the spleen.

To investigate further the role of MCF viruses in M-MuLV–induced disease, we employed a strain of partially congenic Balb/c mice carrying the resistance allele of the *Rmcf* gene (*Rmcf*) developed by Potter et al. (11). The *Rmcf* locus was first described by Hartley et al. (9); *Rmcf* mice are resistant to rapid development of Friend MuLV- (F-MuLV) induced erythroleukemia (12, 13). Fibroblast cell cultures derived from certain (*Rmcf*) strains of mice are resistant to infection by MCFs but not ecotropic MuLVs (9, 14). Resistance is correlated with cell surface expression of an endogenous MCF-related gp70 (SU) protein (12–15). It has been hypothesized that expression of this protein in *Rmcf* mice interferes with spread of in vivo-generated MCFs due to blockage of MCF receptors by the endogenous MCF-related gp70. Indeed, MCFs propagate poorly or not at all in *Rmcf* mice inoculated with F-MuLV (12, 13). The *Rmcf* gene may code for the MCF-related gp70 (16) or, alternatively, for a gene affecting expression of endogenous MuLV-related proviruses (16, 17).

[1] *Abbreviations used in this paper:* M- and F-MuLV, Moloney and Friend murine leukemia virus, respectively; M- and F-MCF, Moloney and Friend mink cell focus-forming virus, respectively; FM-MuLV, a molecularly cloned F-MuLV recombinant containing the long terminal repeat of M-MuLV; LTR, long terminal repeat; *Rmcf*^{r} and *Rmcf*^{s}, resistance and sensitivity alleles, respectively, of the *Rmcf* gene.

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In the experiments described in this report, we tested the effects of M-MuLV in *Rmcf*^{r} mice. Since Friend and Moloney MCFs share the same receptor as measured in interference assays (18), it seemed likely that *Rmcf*^{r} mice would be resistant to in vivo-generation of M-MCF and that they would be relatively resistant to M-MuLV–induced leukemia. Moreover, MCF-driven preleukemic events might not occur in M-MuLV–infected *Rmcf*^{r} animals. We report here that there is significantly less restriction for M-MuLV leukemogenesis and MCF generation and propagation in *Rmcf*^{r} mice than is the case for F-MuLV–inoculated *Rmcf*^{r} mice and that the difference is governed by the LTR. A model to explain this difference is presented.

Materials and Methods

Viruses and Cell Lines. Generation of a molecularly cloned viral stock of M-MuLV was described previously (19). For M-MCF, either Mo-MCF$_1$-1 or MCFMoLTR viral stock was used. Mo-MCF$_1$-1 contains only one copy of the 75-bp direct repeat of the M-MuLV enhancer (20) while MCFMoLTR contains two direct repeats. MCFMoLTR was generated by molecular cloning in which the Xho I-Cla I fragment of the M-MCF$_1$ genome (1.5 kb to 7.6 kb on the proviral map, encompassing the 3′ half of *gag*, all of *pol*, and the SU portion of *env*) was exchanged at the same sites into an infectious M-MuLV provirus clone. Infectious virus was then recovered by transfection of the MCFMoLTR clone into NIH-3T3 cells. Pathology data reported in Fig. 1 are that of MCFMoLTR. The F-MuLV and F-MCF producer cell lines were the kind gift of Sandra Ruscetti (NIH, Bethesda, MD) (12). FM-MuLV (21) was kindly provided by Nancy Hopkins (MIT, Cambridge, MA). Pseudotypic viral stocks were obtained by superinfection of cells producing one MuLV with an MuLV of a different interference group. Viral titers ranged from 10^4 to 10^6 infectious U/ml as determined by reverse transcriptase assay (for pseudotypic mixtures) (22) or UV/XC assay (23) (for ecotropic virus alone). All cells were grown in DMEM plus 10% calf serum.

Inoculation of Mice. Partially congenic Balb/c mice carrying the resistance allele of the *Rmcf* gene (CxD_2) (11, 16) were the generous gift of Sandra Ruscetti. These mice were interbred after the 5th generation backcross of (DBA/2 × Balb/cAnPt)F$_1$ × Balb/cAnPt (16). Neonatal CxD_2 (*Rmcf*^{r}) or Balb/cAnPt (*Rmcf*^{s}) mice were inoculated intraperitoneally with 0.2 ml viral supernatant and sacrificed when moribund.

Southern Blot Analysis. DNA was obtained from tumor splenocytes and thymocytes as described previously (24, 25). Southern blot procedures were performed using Gene Screen Plus (New England Nuclear, Boston, MA), hybridized in 50% deionized formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate at 42°C, followed by two 30 washes at 65°C in 1×SSC + 1% SDS and two 30 washes at room temperature in 0.1×SSC according to manufacturer's specifications. Blots were then exposed to Kodak XAR-5 film at −80°C. Fragments used for P^{32}-labeled random primer probes were as follows: the 700-bp Bam HI-Eco RI fragment from M-MCF$_1$-1 (20); the ~400-bp Ava I-Eco RI fragment from the F-MCF Clavaco clone (kindly provided by Alan Oliff, Merck and Co., Rahway, NJ) (26); the 8.2-kb Eco RI fragment including the entire 1-LTR permuted F-MuLV genome from F-MuLV Clone 57 (27); the 600-bp Eco RI fragment from 86T5 (28), a cDNA clone for the T cell receptor β chain locus.

Results

Disease Induction in Rmcf^r *Mice by M-MuLV.* To investigate the role of MCFs in M-MuLV–induced disease, neonatal Balb/c mice carrying the *Rmcf*^{r} allele from DBA/2 mice (CxD_2) (11, 16) were inoculated intraperitoneally with M-MuLV as described in Materials and Methods. Wild-type Balb/c mice, which are sensitive to infection by MCFs (*Rmcf*^{s}), were inoculated in parallel as controls. CxD_2 mice are the fifth generation backcrosses of Balb/c × (Balb/c × DBA/2) selected for the *Rmcf*^{r} allele; thus, they are partially congenic to Balb/cAn mice. Since F-MuLV- and AKR-derived MCF viruses propagate poorly in mice carrying the *Rmcf*^{r} gene (8, 12, 13, 16), and since these MCFs and M-MCF share the same receptor in NIH-3T3s (18), we expected that M-MCF would be restricted in these mice as well. Theoretically, this would allow us to test whether inhibition of MCF generation changes the time course or disease frequency induced by M-MuLV. Surprisingly, time course of disease was essentially the same for the *Rmcf*^{r} (CxD_2) mice in comparison with *Rmcf*^{s} Balb/c mice as seen in Fig. 1, upper panel. Both *Rmcf*^{r} and *Rmcf*^{s} mice inoculated with M-MuLV died with an average latency of approximately 16 wk. Moribund mice all exhibited enlarged thymus, spleen, and lymph nodes as described previously for M-MuLV–inoculated NFS and NIH Swiss mice (19, 29).

The unexpected sensitivity of the *Rmcf*^{r} mice to M-MuLV leukemogenesis raised the possibility that these mice no longer carried the *Rmcf*^{r} allele. Therefore, we also inoculated (CxD_2) (*Rmcf*^{r}) and Balb/c (*Rmcf*^{s}) mice with F-MuLV. As shown in Fig. 1, lower panel, F-MuLV–inoculated *Rmcf*^{s} mice exhibited an average time to death of 9 wk with 100% dying by 19 wk, whereas 50% of the *Rmcf*^{r} mice were still living at 37 wk. These results were consistent with those observed by others previously for *Rmcf*^{r} and *Rmcf*^{s} mice (13, 16), and confirmed that our CxD_2 mice still contained the *Rmcf*^{r} allele. Thus, M-MuLV disease is not subject to restriction by the *Rmcf*^{r} allele in the *Rmcf*^{r} mice used in these experiments, while F-MuLV disease is.

M-MCF Generation and Propagation in Rmcf^r *Mice.* Since F-MCF was previously shown to propagate poorly or not at all in F-MuLV–inoculated *Rmcf*^{r} mice (12, 13), it was important to determine if the lack of *Rmcf*^{r} restriction for M-MuLV pathogenesis was associated with a lack of restriction for M-MCF propagation. We tested for the presence of M-MCFs in M-MuLV–induced tumors from *Rmcf*^{r} mice by Southern blot analysis as shown in Fig. 2 *A*. An M-MCF provirus resulting from recombination between the input ecotropic M-MuLV and endogenous polytropic sequences would contain a Bam HI site derived from the polytropic genome at the 5′ end of the MCF *env* region and retain the Xba I site in the 3′ LTR of the M-MuLV parent. Therefore, digestion of DNA with Bam HI plus Xba I will yield a unique diagnostic 2.3-kb fragment hybridizable with an HCF *env* probe if an M-MCF provirus is present (5). As shown in Table 1 and Fig. 2 *B*, 8/9 or 89% of tumor DNAs from M-MuLV–inoculated *Rmcf*^{r} mice readily showed the MCF-specific band. Thus, M-MCFs could generate and propagate in

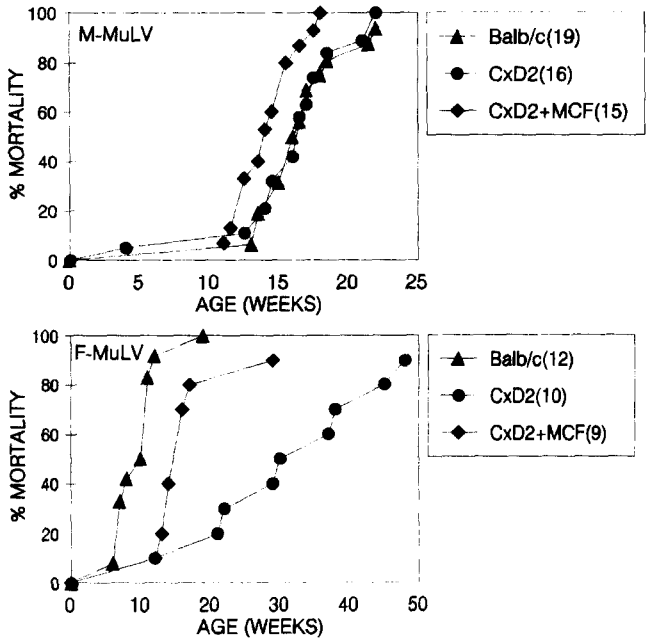


Figure 1. Neonatal CxD_2 (*Rmcf*) or Balb/c (*Rmcf*) mice were inoculated i.p. with M-MuLV or F-MuLV as indicated by legends next to the panels. Number of animals for each inoculum and strain is indicated in parentheses. In addition, a pseudotypic mixture of either M-MuLV/M-MCF (MCFMoLTR) or F-MuLV/F-MCF was inoculated into CxD_2 mice (CxD_2 + MCF). Animals were sacrificed when moribund. Pathogenicity of the different inoculums in the two strains is shown.

M-MuLV-infected *Rmcf* mice. Parallel Southern blot analysis of M-MuLV tumors induced in Balb/c mice indicated that 100% of these tumors were positive for MCF provirus (data not shown).

Table 1. *MCF Provirus in Moribund CxD_2* (Rmcf) *and Balb/c (Rmcf) Mice**

Strain	Virus	No. MCF+/Total	%MCF+
			(%)
CxD2	M-MuLV	8/9	89
Balb/c	M-MuLV	13/13	100
CxD2	Fr-MuLV	0/4	0
Balb/c	Fr-MuLV	3/3	100
CxD2	FM-MuLV	7/9	78
Balb/c	FM-MuLV	7/7	100
CxD2	MuLV/M-MCF	5/5	100
CxD2	Fr-MuLV/Fr-MCF	4/6‡	67‡

* MCF was detected in tumor DNAs by digestion with XbaI + Bam HI for M-MCF and FM-MCF or Eco RI for Fr-MCF followed by Southern blot analysis as described in the Figures and Materials and Methods. r - resistant; s - sensitive.
‡ Diagnostic band barely detectable by Southern blot analysis.

The presence of F-MCFs in *Rmcf* mice has been investigated in previous reports by infectious center assays or by immunoprecipitation of a gPr^{80} MCF envelope polyprotein precursor (12, 13, 16), and the results were negative. It was important to compare the sensitivity of the results obtained by the previous assays with the Southern blot analyses for MCF provirus in Fig. 2. Therefore, we examined *Rmcf* tumors induced by F-MuLV after long latency for the presence of F-MCF. For detection of F-MCF, Eco RI digestion will yield a diagnostic 3.5-kb fragment spanning the *pol* and *env* regions which hybridizes with an F-MCF probe. As seen in Fig. 2 *C*, F-MuLV-induced tumors in CxD_2 mice showed no evidence for MCFs, even though F-MuLV provirus was easily detected by separate Southern analysis using an F-MuLV probe (data not shown). Thus, the Southern blot assay was in agreement with previous assays, and the results for Fig. 2 indicated that M-MCF formation and propagation is not restricted to the same extent as for F-MCFs in *Rmcf* mice. We also examined three tumors induced in Balb/c (*Rmcf*) mice inoculated with F-MuLV. All contained F-MCF provirus (Fig. 2 *D*). These results indicated that failure of F-MCF to generate or propagate in CxD_2 mice was due to the *Rmcf* allele of the *Rmcf* gene, as reported. Furthermore, the *Rmcf* allele may also have provided slight resistance to spread of M-MCF, since one M-MuLV-induced tumor in CxD_2 mice failed to show evidence by this analysis of MCF proviruses.

Genomic Sequences Required for Propagation of M-MCF. The ability of M-MCFs to propagate in *Rmcf* mice in contrast to F-MCFs was quite striking. We wished to determine the regions of the M-MuLV and F-MuLV responsible for the differential generation and/or propagation of the corresponding MCFs in *Rmcf* mice. Even though M-MCF and F-MCF are of the same receptor-binding class with respect to NIH-3T3 fibroblasts (18), it was formally possible that the M-MCF and F-MCF envelope glycoproteins bind MCF receptor(s) with different affinities such that M-MCF is not subject to superinfection resistance by the endogenous MCF-related gp70 on *Rmcf* cells, while F-MCF is. Alternatively, the differing behaviors of F-MCF and M-MCF in *Rmcf* mice could result from differences in LTR enhancer activities. The different pathogenic specificities of F-MuLV and M-MuLV have been mapped to the enhancers in the LTRs (21). To test these hypotheses, we made use of a molecularly cloned recombinant between M-MuLV and F-MuLV, FM-MuLV (30). FM-MuLV contains the U3-R region of the M-MuLV LTR (including the enhancers and promoter) with the remainder of the genome derived from F-MuLV. FM-MuLV induces T lymphoid leukemia, due to the presence of the M-MuLV enhancers (30, 31). FM-MuLV was inoculated into CxD_2 and Balb/c neonatal mice, and the results are shown in Fig. 3. The time course of disease did not vary significantly for FM-MuLV inoculated into *Rmcf* versus *Rmcf* mice as seen in Fig. 3 and, in fact, was similar to that seen for wild-type M-MuLV-inoculated mice. All moribund mice displayed gross pathology typical of M-MuLV-induced T lymphoma, as described above. In addition, T cell receptor beta chain analysis (24, 32) confirmed that tumor cells were of T cell origin (Fig. 4). Southern blot analysis was performed to investigate the pres-

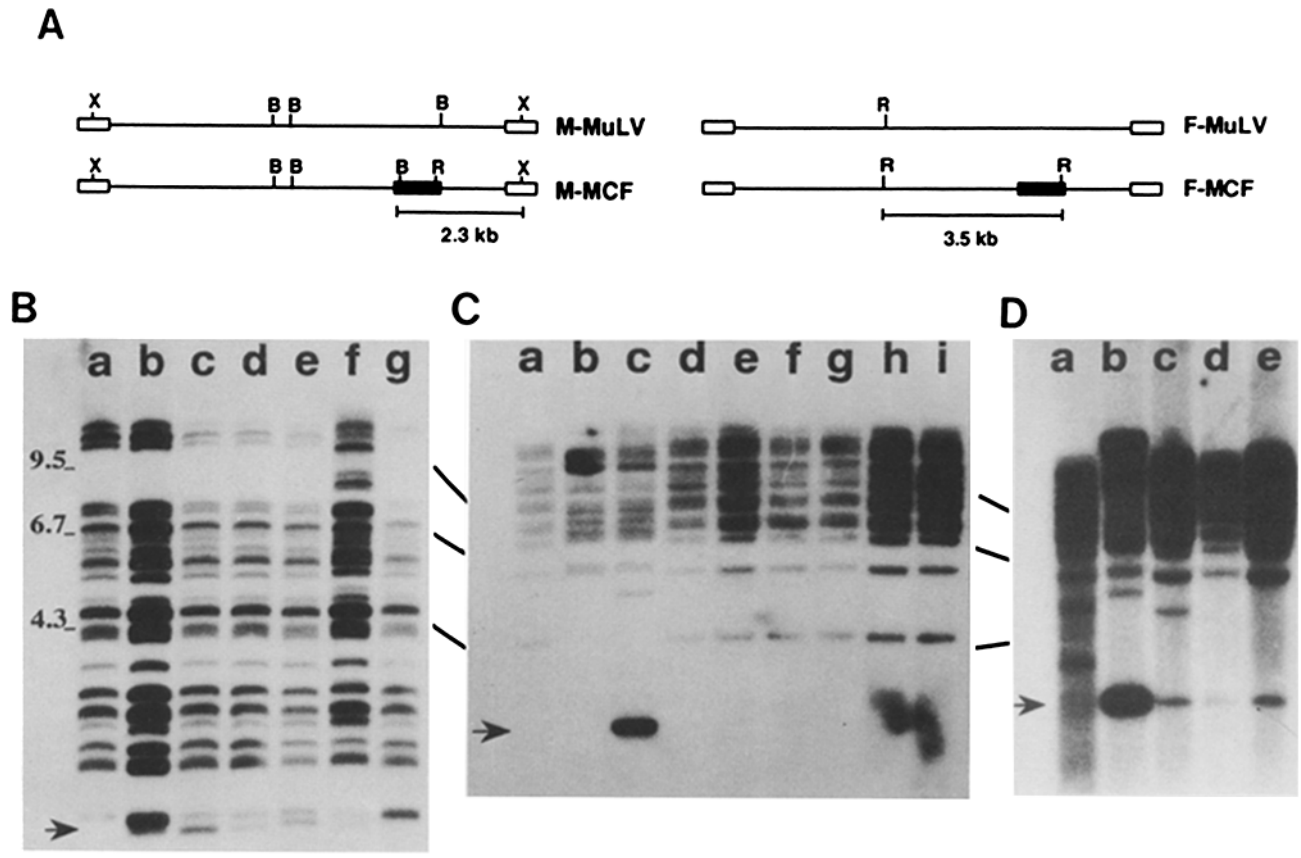


Figure 2. Detection of MCF provirus by Southern blot analysis. (*A*) Restriction maps of M-MuLV, F-MuLV, and expected M- and F-MCFs derived from recombination between the respective inoculated ecotropic virus and endogenous polytropic sequences. M-MCF recombinants are detected by the diagnostic 2.3-kb Bam HI/Xba I fragment of the M-MCF *env* region (5). F-MCF recombinants are detected by a diagnostic 3.5-kb Eco RI fragment of the F-MCF *pol-env* region. These bands detected using an M- or F-MCF–specific probe as described in Materials and Methods. X - Xba I; B - Bam HI; R - Eco RI. (■) - MCF-specific envelope sequences. (*B*) M-MuLV–induced CxD_2 (*Rmcf*) tumor DNAs digested with Xba I plus Bam HI, separated by gel electrophoresis, and analyzed by Southern blot hybridization with an M-MCF–specific probe. Lane *a* - uninoculated CxD_2 mouse DNA; Lanes *b–g* - representative thymic tumor DNAs from six M-MuLV–inoculated moribund CxD_2 mice. Left arrow indicates diagnostic band. (*C*) F-MuLV–induced CxD_2 tumor DNAs digested with Eco RI and hybridized with a F-MCF–specific probe. Lane *a* - uninoculated CxD_2 mouse DNA; Lanes *b* and *c* - F-MuLV and F-MCF producer cell line DNAs, respectively; Lanes *d–g* - thymus and spleen DNAs, respectively, for two F-MuLV–inoculated moribund mice, and lanes *h–i* - spleen tumor DNAs only from two additional mice. (*D*) F-MuLV–induced Balb/c (*Rmcf*) tumor DNAs digested and hybridized as for panel *C*. Lane *a* - Uninoculated Balb/c splenocyte DNA; Lane *b* - F-MCF producer cell line showing expected F-MCF–specific band (*arrow*); Lanes *c–e* - three F-MuLV–induced Balb/c tumor DNAs.

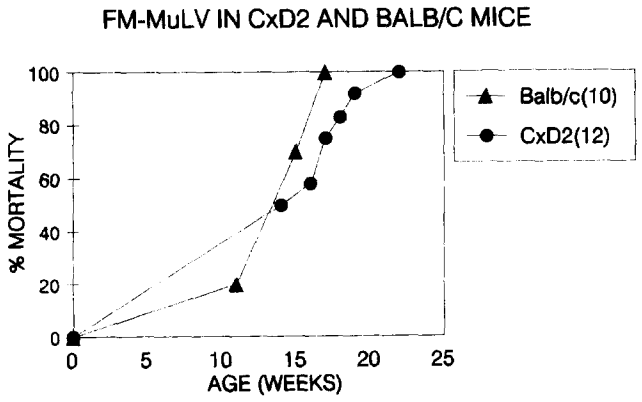


Figure 3. Disease induced by FM-MuLV in CxD_2 and Balb/c mice. FM-MuLV (21) (which contains the U3-R region of M-MuLV and U5 and coding sequences of F-MuLV) was inoculated i.p. into neonatal CxD_2 (*Rmcf*) or Balb/c (*Rmcf*) mice, and animals were sacrificed when moribund. Pathogenicity in the two strains is shown.

ence of MCFs in tumors from FM-MuLV–inoculated mice. An FM-MCF would generate the same diagnostic 2.3-kb Xba I/Bam HI fragment as for M-MCF (Fig. 2), since the FM-MCF would contain the Xba I site in the M-MuLV LTR and the Bam HI site is present in the MCF-specific *env* sequences introduced in the recombination event. Fig. 5 *A* and *B* show that both *Rmcf* and *Rmcf* mice could generate MCF recombinants after inoculation with FM-MuLV. 7/9 *Rmcf* and 7/7 *Rmcf* mice inoculated with FM-MuLV were positive for MCF provirus. These results indicated that the presence of the M-MuLV LTR was sufficient for generation and propagation of MCFs and induction of rapid disease in *Rmcf* mice, even when all other viral determinants were from F-MuLV.

Acceleration of M- or F-MuLV Disease in Rmcf Mice by MCF. It has previously been reported that inoculation of F-MCF pseudotyped with F-MuLV did not cure the defect for F-MuLV-induced early erythroid disease in *Rmcf* mice

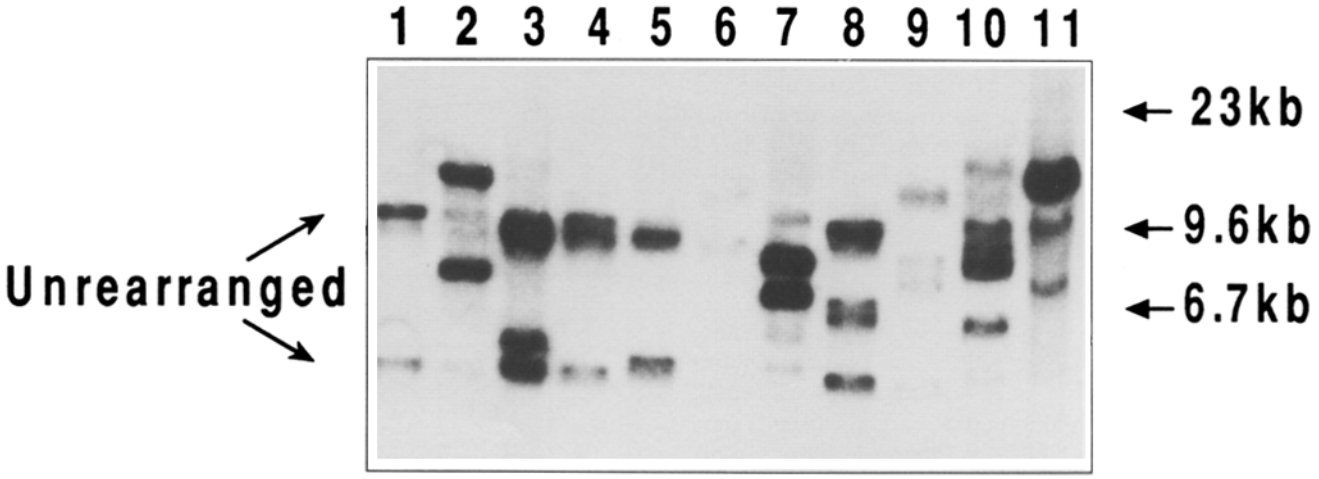
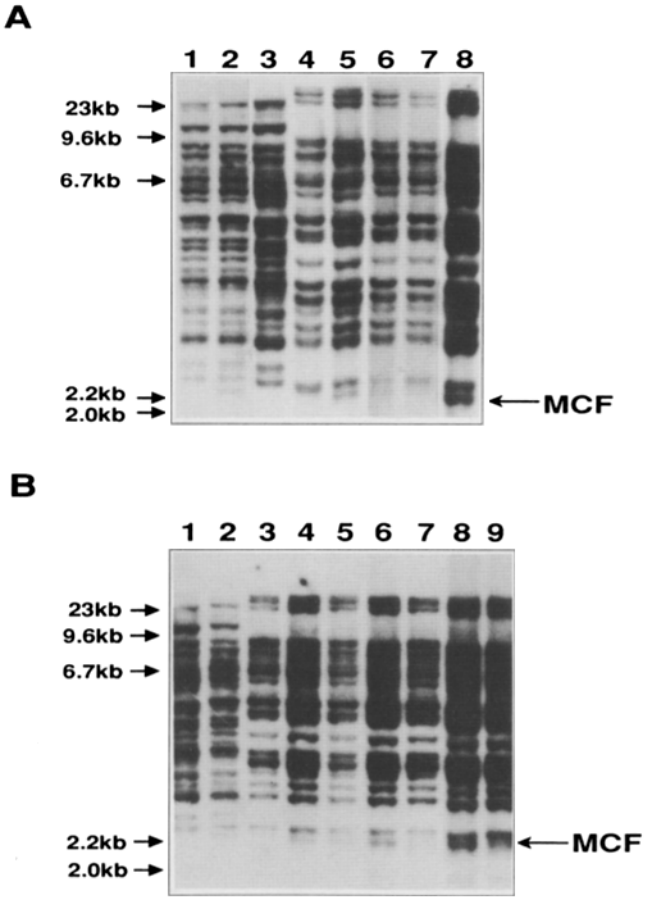


Figure 4. TCR_β gene rearrangement in FM-MuLV-induced tumors. Tumor DNAs were digested with Hpa I and analyzed by Southern blot hybridization with a TCR_β constant region probe (28) as described in Materials and Methods. Lane *1* - uninoculated CxD_2 mouse DNA showing expected germline bands of 11.6 and 6.1 kb; Lanes *2* and *3* - tumor DNAs from FM-MuLV-inoculated CxD_2 (*Rmcf*) mice; Lane *4* - uninoculated Balb/c mouse DNA; Lanes *5–11* - tumor DNAs from FM-MuLV-inoculated Balb/c (*Rmcf*) mice.



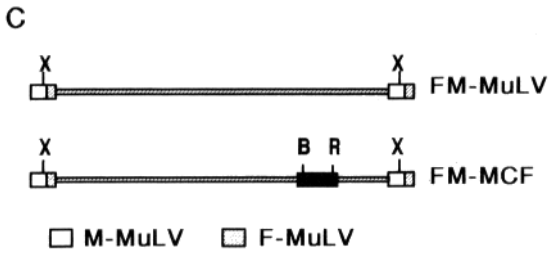


Figure 5. Detection of MCFs in FM-MuLV-induced CxD_2 and Balb/c tumors. DNAs were analyzed by Southern blot hybridization as in Fig. 2 *B* for the M-MCF-specific band using the M-MCF-specific probe (*arrow*). (*A*) Lane *1* - NIH-3T3 cell line DNA; Lane *2* - NIH-3T3-based M-MCF producer cell line (MCFMoLTR); Lane *3* - NIH-3T3-based FM-MuLV producer cell line; Lanes *4–8* - FM-MuLV-induced CxD_2 tumor DNAs. The diagnostic MCF band was present in lanes *5–8*. (*B*) Lanes *1* and *2*- FM-MuLV and MCFMoLTR producer cell line DNAs, respectively, as in panel *A*; Lane *3* - uninoculated Balb/c mouse spleen DNA; Lanes *4–8*- FM-MuLV-induced Balb/c tumor DNAs; Lane *9* - DNA from FM-MuLV-inoculated 8-wk-old Balb/c mouse. (*C*) Restriction maps of FM-MuLV and the expected FM-MuLV MCF arising through recombination of the input virus with endogenous proviral *env* sequences. FM-MuLV contains the Xba I site in its LTR derived from M-MuLV. Thus an FM-MCF would generate the same diagnostic 2.3-kb XbaI/Bam HI fragment as for M-MCF.

(12). However, the potential effect of such a pseudotypic mixture on F-MuLV-induced disease of longer latency has not been investigated. We inoculated neonatal CxD_2 mice with a pseudotypic mixture of either F-MuLV/F-MCF or M-MuLV/M-MCF. Slight acceleration by M-MCF of M-MuLV-induced disease (2–3 wk) was observed in CxD_2 mice (Fig. 1, *upper panel*). For F-MuLV-induced disease in *Rmcf* mice, average time to death was decreased by addition of F-MCF to nearly half—from 30 to 16 wk (Fig. 1, *lower panel*). Gross pathology of the disease induced by the F-MuLV/F-MCF inoculum appeared similar to that of F-MuLV alone in *Rmcf* mice with greatly enlarged spleen and normal or regressed lymph nodes

and thymus. Southern blot analysis on F-MuLV/F-MCF–induced tumor DNAs revealed a faint MCF diagnostic band (indicating low copy number) in 4/6 tumors examined and this band was undetectable in the remaining tumors (not shown). Thus, while F-MCF accelerated F-MuLV–induced leukemia, direct infection by F-MCF of the majority of the resulting tumor cells was apparently not required.

Discussion

In these experiments, the pathogenesis of M-MuLV in mice carrying the *Rmcf*^{r} and *Rmcf*^{s} alleles was compared by infecting CxD_2 vs. Balb/c mice. In contrast to F-MuLV, M-MuLV showed no significant difference in leukemia time course in *Rmcf*^{r} vs. *Rmcf*^{s} mice, and the majority of tumors developing in *Rmcf*^{r} CxD_2 mice showed evidence of MCF proviruses. Thus, M-MuLV was much less sensitive to the *Rmcf*^{r}-mediated restriction in vivo than F-MuLV. These results were initially surprising, since considerable evidence for the involvement of MCF recombinants in M-MuLV pathogenesis has been reported (3, 4, 5, 10). Moreover, since M-MCFs and F-MCFs bind to the same cellular receptor (18), it might seem that leukemogenesis by M-MuLV should be restricted in *Rmcf*^{r} mice to the same degree as F-MuLV. Indeed, this was the initial motivation for our experiments.

The different restrictions of the *Rmcf*^{r} gene for M-MuLV and F-MuLV might result from two possibilities. First, it is possible that F-MCF and M-MCF glycoproteins might have different affinities for the cellular MCF receptor. If F-MCF had a lower affinity than M-MCF, the endogenous MCF-like gp70 expressed in *Rmcf*^{r} cells might competitively block binding of F-MCF virus particles, but not M-MCF particles. A second possibility could be that enhancer differences between M-MuLV and F-MuLV (dictated by the LTRs) mediate the different responses. The experiments with FM-MuLV clearly support the second possibility, since this virus has the structural genes of F-MuLV, but the LTR of M-MuLV, and it is not restricted by the *Rmcf*^{r} gene.

To interpret these results, it is important to consider that at early times, most MCF particles probably also have MCF envelope proteins, i.e., pseudotyping of MCF genomes with ecotropic *env* proteins is probably rare due to the low multiplicities of infection in vivo at this time. (Other experiments support this notion [5].) Thus, MCF viruses would propagate in vivo in "MCF propagator" cells: cells with (*a*) surface MCF receptors (18, 33) and (*b*) transcriptional milieus compatible with the viral LTR of the infecting virus (6, 21, 30, 34, 35) (Fig. 6 *A*). MCF propagators could be either hematopoietic or non-hematopoietic cells.

The differential restriction of F-MuLV and M-MuLV in *Rmcf*^{r} mice could be explained if there existed (*a*) nonequivalent sets of F-MCF vs. M-MCF propagator cells, and (*b*) differential tissue-specific expression of endogenous MCF-like gp70. In particular, for F-MuLV in *Rmcf*^{s} mice, MCF propagators probably include erythroid and myeloid hematopoietic cells, fibroblasts and stromal cells, since the F-MuLV LTR is active in these cell types (9, 12, 27, 36), and in vivo and

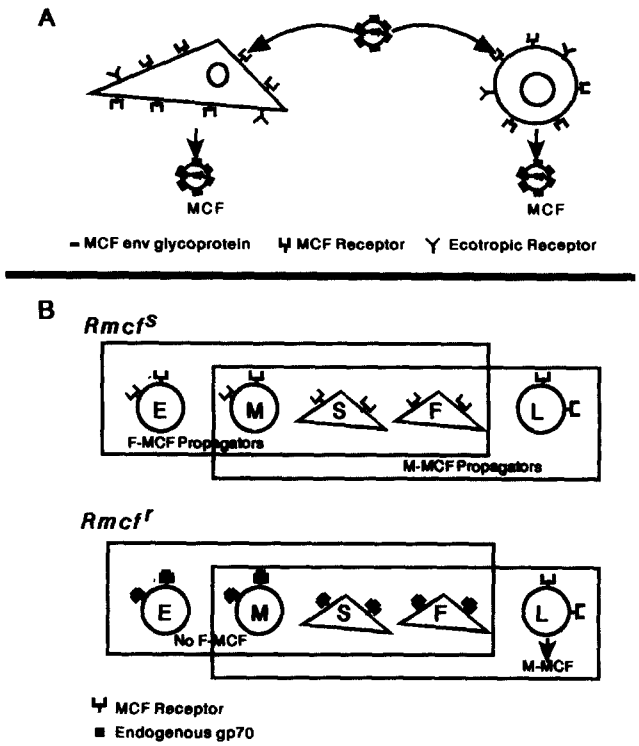


Figure 6. A proposed model for differential in vivo propagation of M-MCF and F-MCF in CxD_2 mice. (*A*) Early stages in propagation of MCF viruses. MCF propagators must have MCF receptors (and potentially ecotropic receptors as well) on their cell surface and the MCF LTR must be active in these cells. Propagators could be adherent or hematopoietic cells. (*B*) Tissue-specific expression of the (*Rmcf*-induced endogenous MCF-like gp70^{env} could explain *Rmcf*-mediated resistance to F-MCF, but not M-MCF (see text). L, M, S, F, and E - lymphoid, myeloid, bone marrow stroma, fibroblast, and erythroid cells, respectively.

in vitro experiments suggest that many of these have surface MCF receptors (9, 15, 16) (Fig. 6 *B*, *upper panel*). If all potential F-MCF propagators are expressing endogenous MCF gp70 in *Rmcf*^{r} mice, they would be blocked for F-MCF infection (Fig. 6 *B*, *lower panel*) and propagation. On the other hand, M-MuLV could generate M-MCFs in *Rmcf*^{r} mice (and cause rapid disease) if at least some M-MCF propagators did not express *Rmcf*-controlled endogenous MCF gp70. Lymphoid cells would be candidates for such cells; they would not be F-MCF propagators in either *Rmcf*^{r} or *Rmcf*^{s} mice because they do not efficiently support transcription from the F-MuLV LTR (37–39) (Fig. 6 *B*, *lower panel*). This model also would explain the behavior of FM-MuLV in *Rmcf*^{r} mice: an FM-MCF would be able to propagate in the same cells that an M-MCF can (e.g., a lymphoid cell), since it contains the same LTR as M-MCF.

Previous results of Buller et al. (15) strongly support the model in Fig. 6. These investigators showed that expression of endogenous MCF-like gp70 in *Rmcf*^{r} mice is found predominantly on erythroid cells and to a lesser extent on myeloid and lymphoid progenitors. Notably, 90–95% of *Rmcf*^{r} mouse thymocytes do not express the endogenous gp70.

While the *Rmcf* gene did not delay the time course of leukemogenesis for M-MuLV or FM-MuLV in CxD_2 mice, there may have been slight blockage in MCF formation, since one tumor did not show evidence for MCFs. According to our model, it is quite likely that some M-MCF propagator cells are blocked in *Rmcf* mice (e.g., myeloid cells). Thus, a decrease in efficiency of MCF formation and propagation for M-MuLV and FM-MuLV in CxD_2 mice would be reasonable.

As shown in Fig. 1, lower panel, a pseudotypic mixture of F-MuLV/F-MCF showed greatly accelerated disease in comparison to F-MuLV alone in *Rmcf* mice. Two aspects require explanation. First, the fact that the F-MCF accelerated the disease implies that it had an effect in vivo, even in *Rmcf* mice. However, since the F-MCF was administered as a pseudotypic mixture with F-MuLV, F-MCF genomes could infect cells by way of ecotropic F-MuLV glycoprotein and ecotropic receptors. The second aspect is that in the rapidly developing tumors induced by F-MuLV/F-MCF, 2/6 tumors did not contain detectable F-MCF provirus, and others showed only low concentrations. Thus, F-MCF may have accelerated the disease course by infecting some cell other than the ultimate tumor cell itself. We have observed a related phenomenon in M-MuLV–induced tumors. As described in the Introduction, M-MuLV induces a preleukemic state of generalized hematopoietic hyperplasia in the spleen which is necessary for rapid disease. However, this does not result from direct M-MuLV infection of hematopoietic progenitors (40). Rather, combined infection of M-MCFs and M-MuLV in other cells (perhaps bone marrow stroma) indirectly leads to preleukemic hyperplasia (10). By the same token, combined F-MuLV/F-MCF infection of nonhematopoietic cells might accelerate leukemogenesis without F-MCF infecting the final tumor cells.

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