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Different Abilities of Friend Murine Leukemia Virus (MuLV) and Moloney MuLV To Induce Promonocytic Leukemia Are Due to Determinants in both Ψ-*gag*-PR and *env* Regions

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Moloney murine leukemia virus (M-MuLV) is capable of inducing promonocytic leukemia in 50% of adult BALB/c mice that have received peritoneal injections of pristane, but Friend MuLV strain 57 (F-MuLV) is nonleukemogenic under similar conditions. It was shown earlier that these differences could not be mapped to the U3 region of the virus long terminal repeat, indicating the probable influence of structural genes and/or R-U5 sequences. In this study, reciprocal chimeras containing exchanged structural genes and R-U5 sequences from these two closely related viruses were analyzed for differences in ability to induce disease. Results showed that two regions of F-MuLV, Ψ-*gag*-PR and *env*, when substituted for those of M-MuLV were dramatically disease attenuating. The 5′-most region, which is widely distributed, overlaps with the 5′ end of the *env* intron and includes the RNA packaging region, Ψ, the entire *gag* coding region, and the viral protease coding region (PR) of *pol*. It was also found that reciprocal constructs having substitutions of both of these regions of M-MuLV in an F-MuLV background allowed full reestablishment of promonocytic leukemia. These leukemias were positive for c-*myb* rearrangements which are characteristic of M-MuLV-induced promonocytic leukemias. Neither region alone, however, was sufficient to produce disease with a greater incidence than 13%. Further studies demonstrated that the inability of viruses with Ψ, *gag*, PR, or *env* sequences from F-MuLV to induce leukemia in this model system was not due to their inability to replicate in hematopoietic tissue, to integrate into the c-*myb* locus early on after infection in vivo, or to express *gag-myb* mRNA characteristic of M-MuLV-induced preleukemic cells and acute leukemia.

Moloney murine leukemia virus (M-MuLV)-induced myeloid leukemia (MML) can be induced with high incidence in adult BALB/c mice, but only when the mice are undergoing a chronic peritoneal inflammation as a result of pristane injection (26, 37, 38). The disease manifests itself at the peritoneal inflammatory site at 3 to 4 months after virus inoculation and is characterized by proliferation of promonocytic leukemia cells. Analysis of leukemias has demonstrated that 100% of them have undergone insertional mutagenesis at the c-*myb* locus (reference 26 and unpublished data).

Since Friend MuLV (F-MuLV) is closely related to M-MuLV but does not induce promonocytic leukemias in adult pristane-treated mice, we have had an ongoing interest in determining the genetic basis for the different in vivo responses of the viruses. In a previous study, we determined that the different abilities of F- and M-MuLVs to induce promonocytic disease could not be attributed to sequences in the U3 region of the long terminal repeat (LTR) (36), even though other studies had shown that the U3 regions of F-MuLV and M-MuLV contain determinants for erythroid and lymphoid leukemia (5, 6, 17, 30).

In this study, we set out to determine which of the genes outside of the U3 region in M-MuLV influence development of promonocytic leukemia in adult mice. Reciprocal chimeras between the two viruses were analyzed for disease induction and the ability to replicate in hematopoietic tissue of adult BALB/c mice. In addition, leukemias which developed in these mice were analyzed for virus integration and rearrangement of the c-*myb* gene. Our results show that two distinct regions, the Ψ-*gag*-PR and *env* regions, contain the determinants that are responsible for the different abilities of F-MuLV and M-MuLV to induce MML in adult BALB/c mice.

MATERIALS AND METHODS

Molecular constructions. Viral DNA chimeras of F-MuLV (22) and M-MuLV 8.2 (28) were prepared from plasmids containing permuted versions of MuLV genomic DNA bearing a single LTR. F-MuLV 57 DNA was cloned in the *Eco*RI site of either pUC19A (30) or Bluescript to obtain p57A and pBS/57, respectively. M-MuLV 8.2 was cloned in the *Hin*dIII site of pUC19B (30), providing p8.2B. These constructs were used to prepare recombinant plasmids pFME/A, pFMP/B, and pFMU5G/A as previously described (23). The designation A or B at the end of a plasmid name refers to pUC19A or pUC19B, respectively. pFMU5GE/A was prepared by ligating the 2.6-kb *Sph*I-*Cla*I fragment of p8.2B containing mostly *env* sequences (Fig. 1) to the 8.4-kb *Sph*I-*Cla*I fragment of pFMU5G/A. The chimeras pMFP/B and pMFE/B contain the 2.4-kb *Bcl*I-*Sph*I and the 2.6-kb *Sph*I-*Cla*I fragments of F-MuLV, respectively, in the M-MuLV 8.2 context. The construct used to generate the virus MFL contains the 0.62-kb *Cla*I-*Kpn*I fragment of F-MuLV 57 in the MuLV 8.2 back-

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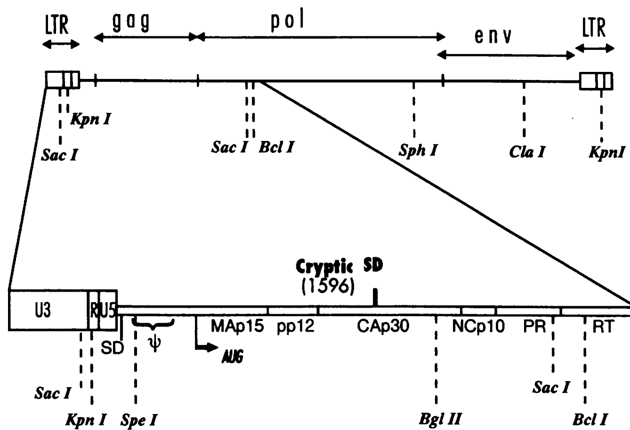

FIG. 1. Features within LTR and *gag* regions of M-MuLV. SD, splice donor used in envelope message processing. Cryptic SD, splice donor used in c-*myb* activation by the retrovirus. *gag* translation begins at AUG shown. *Spe*I and *Bgl*II are restriction endonuclease sites used in preparation of viruses with a chimeric *gag* region.

ground and was described by Chatis et al. (6), who called it MF-3. The construct pMFE/B was described by Sitbon et al. (30), who called it MFM.

The viral chimeras pMFU5SD/B, pMFΨMACA/B, and pMFNCPR/B were constructed as follows. The *Nhe*I-*Sal*I fragment of M-MuLV 8.2, which encompasses all of R, U5, and *gag* and less than half of the *pol* gene, was cloned into pBR327, yielding pMRG, and the similar *Kpn*I-*Eco*RI fragment of F-MuLV57 was cloned into pUC19, yielding pFU5G as previously described (23). These two plasmids were subsequently used to create the intermediate chimeric Friend and Moloney constructions pMRGF(U5SD), pMRGF(ΨMACA), and pMRGF(NCPR) as described below. The 0.24-kb *Kpn*I-*Spe*I fragment of pFU5G was ligated with the 4.4-kb *Kpn*I-*Spe*I and 2.6-kb *Kpn*I-*Kpn*I fragments of pMRG to create pMRGF(U5SD). The 1.6- and 5.6-kb *Spe*I-*Bgl*II fragments of pFU5G and pMRG were ligated to create pMRGF(ΨMACA), and the 0.83- and 6.4-kb *Bgl*II-*Bcl*I fragments of pFU5G and pMRG, respectively, were ligated to create pMRGF(NCPR). pMFU5SD/B, pMFΨMACA/B, and pMFNCPR/B were assembled by ligation of the 4.1-kb *Nhe*I-*Sal*I fragment of the corresponding intermediate constructions with the 6.8-kb *Sal*I-*Nhe*I fragment of p8.2B.

Tissue culture, transfection, and viral stocks. NIH 3T3 fibroblasts were cultivated in Dulbecco's modified Eagle's medium with glutamine (2 mM), penicillin (50 IU ml^{-1}), streptomycin (50 μg ml^{-1}), and 10% heat-inactivated fetal calf serum. Plasmid DNA (6 to 8 μg) was digested with *Eco*RI to excise the permuted retroviral genomic DNA and transfected into NIH 3T3 cells without prior ligation according to the following adaptation of the method described by Golub et al. (18). DNA was dissolved in 10 μl of TE (10 mM Tris [pH 7.5], 1 mM EDTA). Cells were adjusted to a concentration of 5×10^5 cells ml^{-1} in Tris-buffered saline (25 mM Tris HCl [pH 7.4], 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM $NaHPO_4$). Two microliters of the cell suspension and 25 μl of 1 mg of DEAE-dextran ml^{-1} in Tris-buffered saline were added to the DNA solution. After gentle vortexing, the cells were incubated at 37°C. Transfection was monitored by focal immunofluorescence assay (31) and assay of reverse transcriptase activity (16).

Viral stocks prepared from 24-h supernatants were titrated by the UV-XC syncytial plaque assay (24). For the quantitation of the number of bone marrow cells producing viruses (infectious centers), bone marrow cells were plated on NIH 3T3 cells; the number of infectious centers was then determined by the UV-XC plaque assay.

Animal experiments. For determination of viral infectivity and leukemogenicity, 4- to 6-week-old female BALB/cANCr mice (Frederick Cancer Research Facility, Fort Detrick, Frederick, Md.) were injected intraperitoneally with 0.5 ml of 2,6,10,14-tetramethylpentadecane (pristane; Aldrich Chemical Co., Milwaukee, Wis.), and after an additional 10 days to 3 weeks, they were inoculated intravenously with cell culture supernatant containing 5×10^5 PFU of recombinant virus. Mice were monitored for development of leukemia as previously described (37) by preparing smears of peritoneal ascites fluid and staining with Diff-Quik (American Scientific Products, McGaw Park, Ill.). Latency was defined as the first day a positive smear was found for a leukemic mouse.

Southern blot hybridization of genomic DNA. DNA was extracted and analyzed for c-*myb* rearrangement by Southern blot hybridization as previously described (37). DNA was digested with either *Eco*RI or *Bam*HI and hybridized with a nick-translated 1.4-kb genomic mouse c-*myb Bgl*II fragment that contains exon 5(vE2) (26).

Preparation of total RNA. Total RNA was prepared by the method of Chomczynski and Sacchi (9). Spleen, bone marrow, and granuloma were each resuspended in guanidinium thiocyanate extraction buffer as whole organs and then divided into 0.5-ml aliquots for further preparation of RNA.

Reverse transcription and amplification of RNA (RT-PCR). Reverse transcription of RNA into cDNA and amplification were carried out by a method based on published procedures of Kawasaki et al. (19) and Ferre and Garduno (15). Briefly, 5 μg of RNA in a 5-μl volume was mixed with 1 μl containing 20 pmol of the 3′ negative-strand primer (PCR primer 1B) (see below) and incubated at 70°C for 10 min. This mixture was then incorporated into a total reaction mixture of 20 μl containing 200 U of M-MuLV reverse transcriptase (GIBCO/BRL), 20 U of RNasin (Promega), 0.01 M dithiothreitol, and 1 mM each deoxynucleoside triphosphate (dNTP). After incubation at 37°C for 45 min, the mixture was diluted to 50 μl, and additional reagents were added to give final concentrations of 1× PCR buffer (Perkin-Elmer), 1 μM each primer, M1A or F1A (the prefix M or F indicates whether *gag* sequences were from M-MuLV or F-MuLV, respectively) and 1B, and 0.5 U of *Taq* polymerase (Perkin-Elmer). A second amplification using nested primers was done in 50 μl by using 5 μl of the first reaction mixture; final concentrations were 1 μM primer M2A or F2A and 2B, 100 μM each dNTP, and 0.5 U of *Taq* polymerase. Amplification reaction mixtures were overlaid with mineral oil and subjected to thermal cycling in a programmable thermal cycler (MJ Research, Cambridge, Mass.). The program included an initial 94°C incubation for 30 s, followed by a three-step cycle that was repeated 25 times and consisted of a 94°C denaturation step for 1 min, a 55°C annealing step for 1 min, and a 72°C elongation step for 1 min. A final incubation was at 72°C for 10 min. Every set of PCR included a negative control that had no template added and a positive control for which MML tumor cell line RNA was used as a template. Southern blot analysis of PCR products was performed as follows. Fifteen microliters from each PCR was electrophoresed on a 4% gel (3% NuSieve [FMC] and 1% agarose [GIBCO/BRL]). Gels were blotted and hybridized by standard techniques (25). Oligonucleotides MJEX-3, FJEX-3, MJEX-4, and FJEX-4 were 5′ end labeled and used separately to probe blots. Oligonucleotides prefixed with M were used for tissues

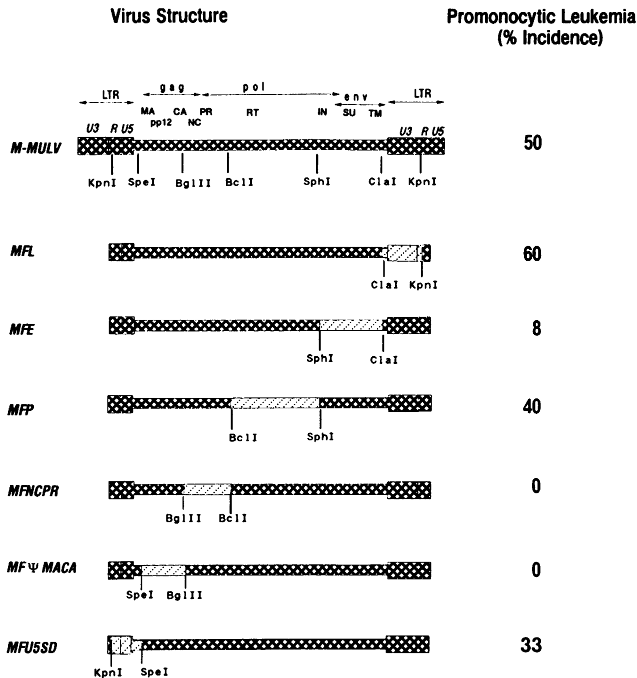

FIG. 2. Structures of chimeric viral genomes with M-MuLV background and F-MuLV substitutions. Open bars, F-MuLV sequences; closed bars, M-MuLV sequences. The overall disease incidence for each virus is presented on the right.

TABLE 1. Attenuation of M-MuLV-induced leukemia by substitution with F-MuLV sequences

Virus[a]	IC[b]/2 × 10^6 bone marrow cells	No. of mice with disease/total no. of mice[c]	Avg latency (days)	c-*myb* rearrangement[d]
M-MuLV[e]	4,930	25/50	85	7/7
	3,800			
	20,800			
	19,500			
MFL[e]		15/25	113	7/9
MFE	29,890	2/25	89	1/1
	3,150			
	32,000			
	5,300			
MFP	69,170	10/25	128	3/3
	168,000			
	282,000			
MFNCPR	4,000	0/20		
	1,800			
	2,700			
MFΨMACA	>25,000	0/20		
	3,300			
	1,300			
	2,400			
MFU5SD	3,000	8/24	111	2/2
	12,500			
	49,000			
	650			

[a] Genomic structures are depicted in Fig. 2.
[b] IC, infectious centers. Bone marrow cells were harvested between 2 and 4 weeks after virus inoculation.
[c] Mice were injected with pristane and 3 weeks later inoculated intravenously with virus. The method of diagnosis is described in Materials and Methods.
[d] Number of leukemias with c-*myb* rearrangements per number evaluated.
[e] Disease incidence data and c-*myb* rearrangements for M-MuLV and MFL were previously published (26, 36, 37).

from mice infected with viruses that had *gag* regions from M-MuLV, and those prefixed with F were used for tissues from mice infected with F-MuLV *gag*.

Synthetic oligonucleotides. Oligonucleotides used for amplification and hybridization were prepared on an Applied Biosystems DNA synthesizer (model 381A). All sequences are shown 5′ to 3′. PCR primers were as follows: M1A, GGACTCTGCTGACCGG *gag* sense (nucleotides [nt] 1444 to 1459) (27); F1A, GGACCCTGCTGACGGG *gag* sense (nt 1445 to 1460) (GenBank accession no. X02794); 1B, CAGAC CAACGCTTCGGACC *myb* antisense (nt 595 to 613) (2); M2A, CCAATGAAGTCGATGCCGC *gag* sense (nt 1534 to 1552) (27); F2A, CCAATGACATTAATGATGC *gag* sense (nt 1535 to 1553) (GenBank accession no. X02794); and 2B, GATGAGTTCAGGGTTCAGCAC *myb* antisense (nt 517 to 537) (2). All of the following JEX oligonucleotides are *myb* antisense sequences and were used to hybridize blots containing RT-PCR products: MJEX-3, GCTTCTCATCCTGCCT GGG (nt 1588 to 1596 [27]; nt 406 to 415 [2]); FJEX-3, GCTTCTCATCCTCGTTGGG (nt 1589 to 1597 [GenBank accession no. X02794]; nt 406 to 415 [2]); MJEX-4, TGTCCG GTTCTGCCTGGG (nt 1588 to 1596 [27]; nt 478 to 486 [2]); and FJEX-4, TGTCCGGTTCTCGTTGGG (nt 1589 to 1597 [GenBank accession no. X02794]; nt 478 to 486 [2]).

RESULTS

Attenuation of MML by substitution of distinct regions of F-MuLV. F-MuLV is nonleukemogenic for the first 6 months after virus inoculation in adult BALB/c mice. To determine which genetic regions of F-MuLV are unable to support disease, specific regions of the M-MuLV genome were replaced with analogous regions of F-MuLV, and the resulting chimeras were evaluated for infectivity and leukemogenicity. The viruses used in this study are depicted in Fig. 2. The MFL virus, which has an U3 LTR derived from F-MuLV and its remaining genome from M-MuLV, was previously reported and is as leukemogenic as M-MuLV (36). Chimeric viruses constructed for this study had F-MuLV substitutions in the *env* region (MFE), *pol* region (MFP), sequences encoding the *gag* nucleocapsid (NC) and protease (PR) (MFNCPR), the RNA encapsidation region (Ψ) and sequences encoding the matrix (MA), pp12, and capsid (CA) proteins of *gag* (MFΨMACA), and R-U5 and the leader sequence including the splice donor site (MFU5SD). The approximate locations of restriction endonuclease sites used in the viruses for recombination are depicted in Fig. 1. All of the viruses were able to replicate in hematopoietic tissues in vivo, as monitored by infectious center assays using bone marrow cells taken 2 to 4 weeks after virus infection. As shown in Table 1, the average number of infectious centers for each virus was greater than 2,500 per 2 × 10^6 bone marrow cells. The leukemogenic potential of these viruses was determined by inoculating intravenously 5 × 10^5 PFU of virus into pristane-treated adult BALB/c mice, and mice were examined for up to 6 months after virus infection for evidence of promonocytic leukemia. The disease incidences shown in Fig. 2 and Table 1 for M-MuLV and MFL were previously reported (26, 36) and are presented here for comparison. It was found from this study that the *env* sequence from F-MuLV was attenuating, giving an MML incidence of only 8% (two tumors in 25 injected mice), compared with an incidence of 50% for M-MuLV (Fig. 2 and Table 1). In addition, *gag* sequences from F-MuLV had an even more potent negative effect on disease induction, since two chimeric

TABLE 2. Establishment of promonocytic leukemia in F-MuLV by substitution with M-MuLV sequences

Virus[a]	IC[b]/2 × 10^{6} bone marrow cells	No. of mice with disease/total no. of mice[c]	Avg latency (days)	c-*myb* rearrangement[d]
F-MuLV[e]	10,900	0/50		
	2,500			
	970			
	13,500			
FML[e]		0/23		
FME	>6,200	1/25	104	0/1
	5,100			
FMP	>25,000	0/25		
	25,000			
	11,800			
FMU5G	>25,000	6/45	122	4/4
	>25,000			
	>25,000			
FMU5GE	62,000	16/25	123	4/5
	2,900			
	1,200			
	20,300			
	1,930			

[a] Genomic structures are depicted in Fig. 4.
[b] IC, infectious centers. Bone marrow cells were harvested between 2 and 4 weeks after virus inoculation.
[c] Mice received pristane and virus as described in footnote of Table 1, footnote c.
[d] Number of leukemias with c-*myb* rearrangements per number evaluated.
[e] Data on disease incidence for F-MuLV and FML were previously published (36).

viruses, MFNCPR and MFΨMACA, which contain sequences from two different regions of *gag* were unable to induce MML. In contrast, substitution of *pol* or R-U5-splice donor sequences of F-MuLV for those of M-MuLV allowed significant induction of MML (Fig. 2 and Table 1).

DNAs from leukemias induced by the chimeras were examined for rearrangements at the c-*myb* locus because this has been a characteristic of 100% of leukemias induced by M-MuLV (26). For each virus construct, the overall number of tumors that were positive for c-*myb* rearrangements per number tested is reported in Table 1, and examples of the rearrangements are shown in Fig. 3. DNAs depicted in Fig. 3 had rearrangements of a 4.2-kb *Eco*RI fragment, which is the most typical rearrangement observed in M-MuLV-induced promonocytic leukemias.

Establishment of promonocytic leukemia in F-MuLV by substitutions of sequences of M-MuLV. Our results indicated that Ψ-*gag*-PR and *env* sequences of F-MuLV, when placed in the context of the M-MuLV genome, attenuate promonocytic disease in adult BALB/c mice, whereas the LTR and *pol* sequences do not. Reciprocal chimeras were prepared to determine the regions of M-MuLV capable of conferring MML-inducing capabilities in an F-MuLV background. The genetic structure of the viruses used in this experiment are depicted in Fig. 4. F-MuLV as well as the chimeric viruses constructed for this study were tested for infectivity in hematopoietic tissue and were found to be positive (Table 2). The incidence and latency of promonocytic disease for this set of viruses is presented in Table 2, and the overall percentage of disease incidence is given in Fig. 4. As previously reported (36), neither F-MuLV nor FML induced promonocytic leukemia. The next set of viruses, FME, FMP, and FMU5G, had major substitutions which included large segments of M-MuLV *env*, *pol*, or R-U5-*gag*, respectively. Only one mouse developed

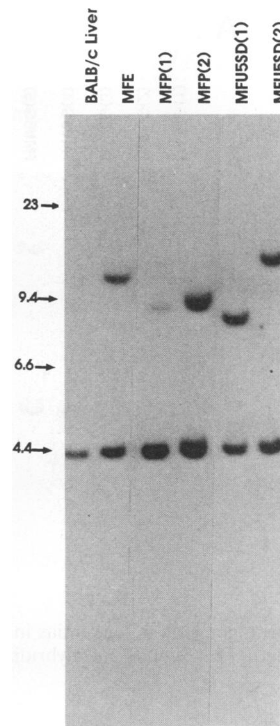

FIG. 3. c-*myb* rearrangements in leukemias induced by viruses having M-MuLV genomic backgrounds and F-MuLV substitutions. DNAs were digested with *Eco*RI, and blots were hybridized with a *myb* probe. Sizes are indicated in kilobases.

leukemia after infection with FME, and it was shown to have no rearrangement at the c-*myb* locus (Fig. 5). No leukemias were induced by FMP. Six leukemias were induced by FMU5G, all of which were positive for c-*myb* rearrangements (Table 2 and Fig. 5); however, the incidence was only 13%, compared with 50% for M-MuLV. These results demonstrated

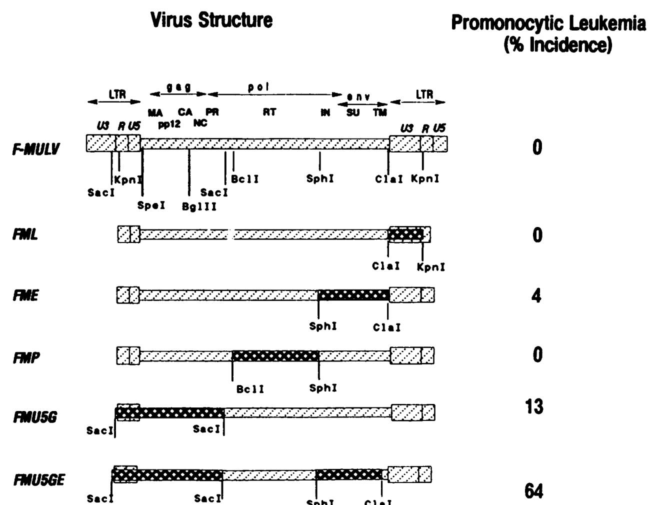

FIG. 4. Structures of chimeric viral genomes with the F-MuLV background and M-MuLV substitutions. Open bars, F-MuLV sequences; closed bars, M-MuLV sequences. The overall disease incidence for each virus is presented on the right.

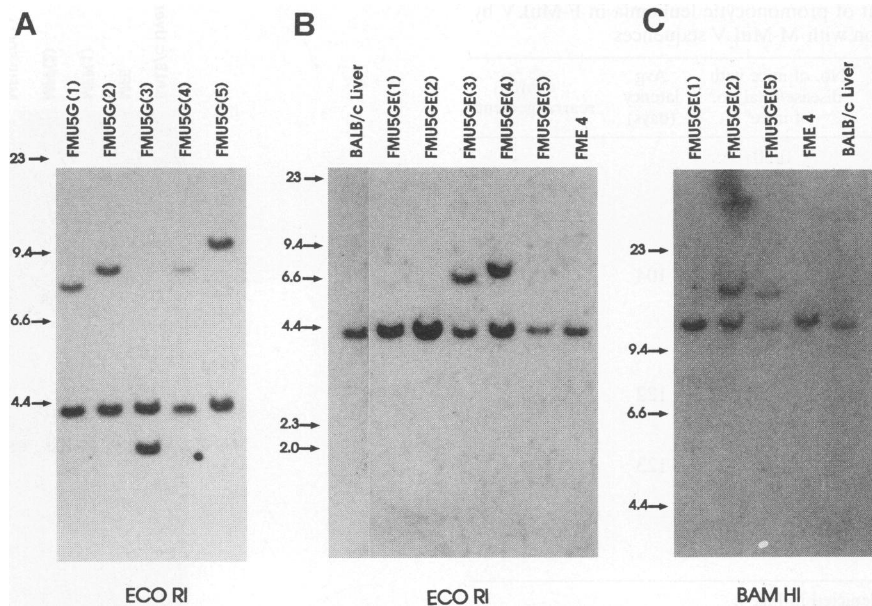

FIG. 5. c-*myb* rearrangements in leukemias induced by viruses having the F-MuLV genomic background with M-MuLV substitutions. DNAs were digested with *Eco*RI or *Bam*HI and hybridized with a *myb* probe. Sizes are indicated in kilobases.

that no chimeras with single regions of substitution could mimic the wild-type virus. We next tested the effect of substitution of the two regions of M-MuLV, Ψ-*gag*-PR and *env*, which conferred partial MML-inducing ability, into an F-MuLV context. The corresponding virus, FMU5GE (Fig. 4), produced a very high incidence of disease (64%), and four of five FMU5GE-induced leukemias examined for c-*myb* rearrangement were positive (Table 2 and Fig. 5). This finding confirmed the results shown in Fig. 2 and Table 1 on attenuation of M-MuLV by F-MuLV sequences, which suggested that these two regions of F-MuLV located outside the LTR were entirely responsible for its lack of MML-inducing ability.

Insertional mutagenesis of c-*myb* after virus infection. Since no correlation was observed between the ability of F-MuLV, M-MuLV, or chimeric viruses to replicate in hematopoietic tissue and cause disease (Table 2), we began to look for other potential blocks in the disease process of the non-MML-inducing viruses. Previous analysis of M-MuLV-induced tumors had indicated that the formation of *gag-myb* mRNA is required as a part of the activation mechanism of c-*myb* in M-MuLV-induced tumors and that a specific donor splice site is utilized in *gag* to produce this RNA (26). In addition, we had previously demonstrated that in mice inoculated with M-MuLV, *gag-myb* mRNA produced as a result of insertional mutagenesis of the c-*myb* locus could be detected in preleukemic mice by RT-PCR as early as 2 to 3 weeks after virus infection (21). Since *gag* functions in proto-oncogene activation and *gag* is in part responsible for the nonpathogenic nature of F-MuLV, one explanation for the inability of viruses containing *gag* sequences from F-MuLV to cause disease might be their inability to support the formation of the *gag-myb* mRNA. Therefore, we examined bone marrow, spleen, and inflammatory granuloma tissue, caused by pristane injection, by RT-PCR for the presence of *gag-myb* fusion message. Total RNA was extracted from tissues obtained from mice infected for 4 to 8 weeks with M-MuLV, F-MuLV, or several chimeric viruses. The RNA was subjected to reverse transcription, and two rounds of amplification were performed with nested primers homologous to *gag* and *myb*. Products from the second round of amplification were separated on agarose gels, and Southern blots derived from these products were hybridized with oligonucleotide probes specific for three different *gag-myb* junctions. The JEX-3 and JEX-4 probes detected splice junctions of *gag* to *myb*, exons 3 and 4, respectively. These are the junctions found to be present in all M-MuLV-induced promonocytic leukemias examined so far. The results in Table 3 demonstrated that both F-MuLV- and M-MuLV-inoculated mice were positive for the leukemia-specific messenger. All of 12 F-MuLV-inoculated mice were positive in at least one tissue (this includes data from tissue samples taken both 4 and 8 weeks after virus infection). All of these mice were positive with the FJEX-4 probe and usually at multiple sites; in addition, three mice were positive with the FJEX-3 probe. Figure 6 depicts PCR products in F-MuLV-infected mice after hybridization with FJEX-4, which demonstrates the spliced mRNA product which is most commonly observed in M-MuLV-induced leukemias. As shown in Table 3, mice inocu-

TABLE 3. Number of virus-infected mice positive in RT-PCR analysis for *gag-myb* message

Virus	No. of mice positive for *gag-myb* mRNA[a]/total no. tested	
	4 wk	8 wk
M-MuLV	5/6	5/6
MFE	4/6	0/6
MFNCPR	5/6	1/6
MFΨMACA	6/6	4/6
F-MuLV	6/6	6/6
FME	6/6	4/6
FMP	6/6	6/6
FMU5GE	2/3	3/3

[a] Number of mice positive for *gag-myb* RNA in one or more tissues (bone marrow, spleen, and peritoneal granuloma resulting from pristane injection) with the JEX-3 or JEX-4 probe.

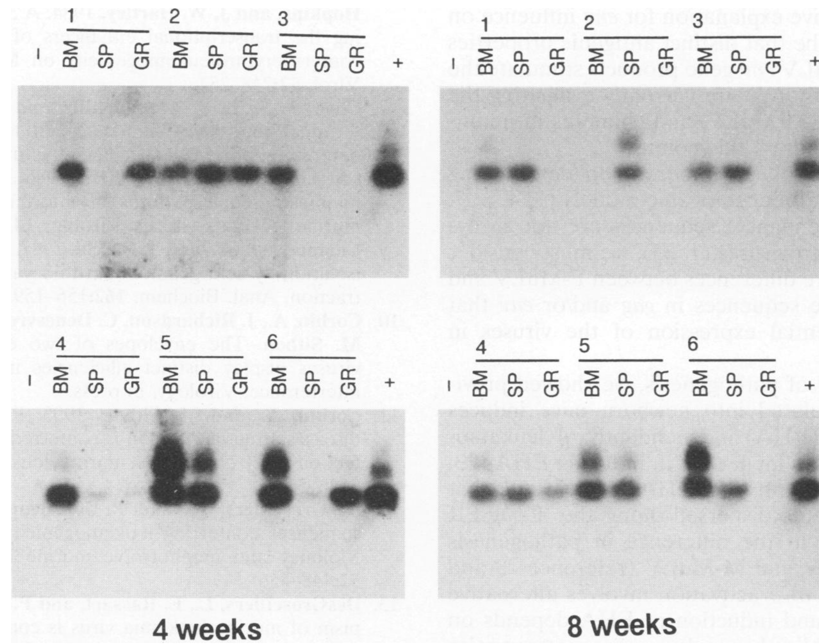

FIG. 6. Detection of *gag-myb* fusion mRNA in tissues of F-MuLV-infected mice. Mouse tissues were examined either 4 or 8 weeks after virus inoculation. Hybridization was carried out with the FJEX-4 probe. BM, bone marrow; SP, spleen; GR, granuloma.

lated with a number of other chimeras, with and without F-MuLV-derived *gag*, were also strongly positive for *gag-myb* mRNA. A comparison of the tissue distribution of *gag-myb*-positive cells in F-MuLV- and M-MuLV-infected mice is presented in Table 4. Since there were no tissues for which M-MuLV-infected mice were demonstrated to be more positive than F-MuLV-infected mice, it was concluded that a difference in tissue distribution of the leukemia-specific message is not likely to account for the difference in pathogenicity of the two viruses.

DISCUSSION

This study and one reported previously demonstrate that clearly distinct determinants of F-MuLV are responsible for its lack of induction of MML in adult-inoculated animals and T lymphomas in newborn-inoculated animals. Whereas it had been shown, using M-MuLV/F-MuLV chimeras, that the U3 region of M-MuLV is necessary for lymphoid disease (5, 17), our previous studies showed that sequences independent of the LTR of M-MuLV are necessary for promonocytic leukemia. In fact, reciprocal M-MuLV/F-MuLV chimeric viruses that exchange the U3 region cause either lymphomas or promonocytic leukemias but not both (36). The results of the present study further define the determinants of M-MuLV specifically required for inducing promonocytic leukemia in adult BALB/c mice. These are found in two separate regions, Ψ-*gag*-PR and *env*. When M-MuLV substitutions including both of these regions were present in a backbone of F-MuLV, it was possible to reestablish a high incidence of induction of promonocytic leukemia by F-MuLV. When individual M-MuLV Ψ-*gag*-PR or *env* regions were substituted for analogous regions of F-MuLV, however, wild-type pathogenicity was never obtained; individual substitution of either of these regions did not produce a disease incidence that exceeded 13%.

When our present studies implicated an important role of M-MuLV *gag* in the development of promonocytic leukemias involving c-*myb*, we envisioned that this region might be important in the alternative splicing from *gag* to *myb* observed in M-MuLV-induced promonocytic leukemias (21, 26). Furthermore, we proposed that F-MuLV might be defective in promoting this splicing. However, when we examined F-MuLV-infected mice early on for the presence of *gag-myb* mRNA, we were able to detect the fusion message with at least equal frequency as we detected this mRNA in M-MuLV-infected mice. Nevertheless, it remains possible that in F-MuLV-infected animals, *gag-myb* RNA products are not effective in producing a functionally active *gag-myb* protein or that the alternatively spliced RNA is not expressed in the specific myeloid or precursor target cells that are required for leukemogenesis.

Since we found that envelope influences the lack of induction of MML by F-MuLV, it is possible that differences in Env can affect cell distribution in vivo. Several studies have described a difference in ecotropic receptor recognition between F-MuLV and M-MuLV in a mouse cell line (8, 14), and recently we have shown that F-MuLV and M-MuLV may present different interference patterns in vivo, which suggested different receptor recognition according to the cell compart-

TABLE 4. Tissue distribution *gag-myb* positive cells in mice infected with F-MuLV or M-MuLV

Virus	Time (wk) after virus inoculation	No. of mice positive for *gag-myb* mRNA[a]/total no. tested)		
		Bone marrow	Spleen	Granuloma
F-MuLV	4	5/6	4/6	3/6
	8	6/6	5/6	5/5
M-MuLV	4	1/6	4/6	4/6
	8	1/6	5/6	2/6

[a] Number of mice having PCR products positive with the JEX-3 or JEX-4 probe.

ment (10, 11). An alternative explanation for *env* influence on leukemia induction might be that distinct antigenic properties of the F-MuLV and M-MuLV *env* gene products stimulate the immune response differently. We are currently evaluating the pathogenicity of F-MuLV, M-MuLV, and chimeras in immunocompromised mice to address this point.

Enhancer sequences have been important in determining disease specificity in several leukemogenic models (3, 4, 6, 7, 12, 13, 33, 34, 39). Since enhancer sequences are not always confined to the LTR of retroviruses (1, 35), we must consider the possibility that there are differences between F-MuLV and M-MuLV in enhancer-like sequences in *gag* and/or *env* that could account for differential expression of the viruses in cellular subsets.

Using a different model of pathogenesis, we showed previously that F-MuLV inoculated into newborn mice induces severe hemolytic anemia (EHA) independently of leukemogenic ability and M-MuLV is ineffective in inducing EHA (23, 30, 32). Interestingly, in both the EHA model and that described here, determinants dispersed along the Ψ-*gag*-PR region play a major role in the difference in pathogenesis observed between F-MuLV and M-MuLV (reference 23 and this study). The facts that *myb* activation involves alternative splicing from this region and induction of EHA depends on efficient formation of the spliced *env* subgenomic message raise the interesting possibility that the opposite virulence of F-MuLV and M-MuLV in the MML and EHA models could be due to cell-specific regulation of splicing influenced by the Ψ-*gag*-PR region. In favor of this view is the description of a negative regulatory sequence for splicing in the capsid encoding region of Rous sarcoma virus (20). Therefore, the Ψ-*gag*-PR region of M-MuLV is implicated in both enhancement of MML and attenuation of EHA, whereas the same region of F-MuLV has the opposite influence. Further studies on these models are likely to provide more insight on the precise mechanisms by which these sequences influence pathogenesis in the promonocytic and erythroid models.

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