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Characterization of Epitopes Defining Two Major Subclasses of Polytropic Murine Leukemia Viruses (MuLVs) Which Are Differentially Expressed in Mice Infected with Different Ecotropic MuLVs

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Polytropic murine leukemia viruses (MuLVs) arise in mice by recombination of ecotropic MuLVs with endogenous retroviral envelope genes and have been implicated in the induction of hematopoietic proliferative diseases. Inbred mouse strains contain many endogenous sequences which are homologous to the polytropic *env* genes; however, the extent to which particular sequences participate in the generation of the recombinants is unknown. Previous studies have established antigenic heterogeneity among the *env* genes of polytropic MuLVs, which may reflect recombination with distinct endogenous genes. In the present study, we have examined many polytropic MuLVs and found that nearly all isolates fall into two mutually exclusive antigenic subclasses on the basis of the ability of their SU proteins to react with one of two monoclonal antibodies, termed Hy 7 and MAb 516. Epitope-mapping studies revealed that reactivity to the two antibodies is dependent on the identity of a single amino acid residue encoded in a variable region of the receptor-binding domain of the *env* gene. This indicated that the two antigenic subclasses of MuLVs arose by recombination with distinct sets of endogenous genes. Evaluation of polytropic MuLVs in mice revealed distinctly different ratios of the two subclasses after inoculation of different ecotropic MuLVs, suggesting that individual ecotropic MuLVs preferentially recombine with distinct sets of endogenous polytropic *env* genes.

Mice which harbor endogenous ecotropic murine leukemia viruses (MuLVs) and mice inoculated with exogenous ecotropic MuLVs frequently generate host range variants termed polytropic MuLVs (19, 20, 25, 27, 36, 42, 43, 50). The variants are infectious for mouse cells as well as cells of several heterologous species (24, 27). Polytropic MuLVs are *env* gene recombinants of the ecotropic MuLVs with endogenous retroviral sequences (4, 7, 8, 11, 15–17, 40, 44) and are thought to be involved in the induction of hematopoietic proliferative diseases. They are also referred to as dualtropic MuLVs (24) or mink cell focus-inducing viruses (MCFs) (27).

The genomes of inbred mice contain many sequences which are closely homologous to the nonecotropic *env* gene sequences of polytropic MuLVs (3, 38, 48, 49); however, the extent to which particular endogenous sequences actually participate in recombination to generate polytropic MuLVs is not precisely known. All polytropic MuLVs have undergone substitution of at least the 5′ region of the *env* gene, which encodes the receptor-binding region of the SU protein (2, 39). This region frequently exhibits a small degree of sequence heterogeneity among different polytropic MuLV isolates (1, 5, 29, 31, 52), which, in some cases, may result from mutations subsequent to recombination. It has been demonstrated, however, that some heterogeneity among polytropic MuLV *env* genes is due to recombination with distinct endogenous retroviral sequences (20).

We have previously described a number of monoclonal antibodies (MAbs) which are specifically reactive with polytropic envelope proteins. The antibodies were able to distinguish many polytropic MuLV isolates (9, 12, 20), and it seemed plausible that some of the observed antigenic heterogeneity might reflect recombination with distinct endogenous polytropic *env* genes. Two of the antibodies (MAb 516 and Hy 7) used in our analyses appeared to react with distinct sets of the polytropic MuLVs we had examined (20, 22). In the present study, we have extended this observation to many polytropic MuLV isolates and found that MAb 516 and Hy 7 define two mutually exclusive antigenic subclasses. We have analyzed chimeric and mutant MuLVs to determine if reactivity to the antibodies depends on alternative allelic gene sequences. Furthermore, we have quantified the polytropic MuLVs from mice infected with different ecotropic MuLVs to determine the frequency of occurrence of the two subclasses of polytropic MuLVs in vivo.

MATERIALS AND METHODS

Cells, viruses, and mice. Mink lung fibroblasts (ATTC CCL-64) (28), mouse SC-1 cells (26), NIH 3T3 cells, and *Mus dunni* (32) cells were used for propagation and assays of MuLVs and for transfection of cloned MuLV proviruses as indicated in the text. The polytropic MuLV, MCF 247 (13), was obtained as a molecularly cloned provirus in pBR322 (30). MCF 13 (13) was obtained from J. Hartley (Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH], Bethesda, Md.). The ecotropic MuLVs, Friend (F)-MuLV$_{57}$ (37) and Moloney (Mo)-MuLV$_{1387}$ (21), derived from transfected

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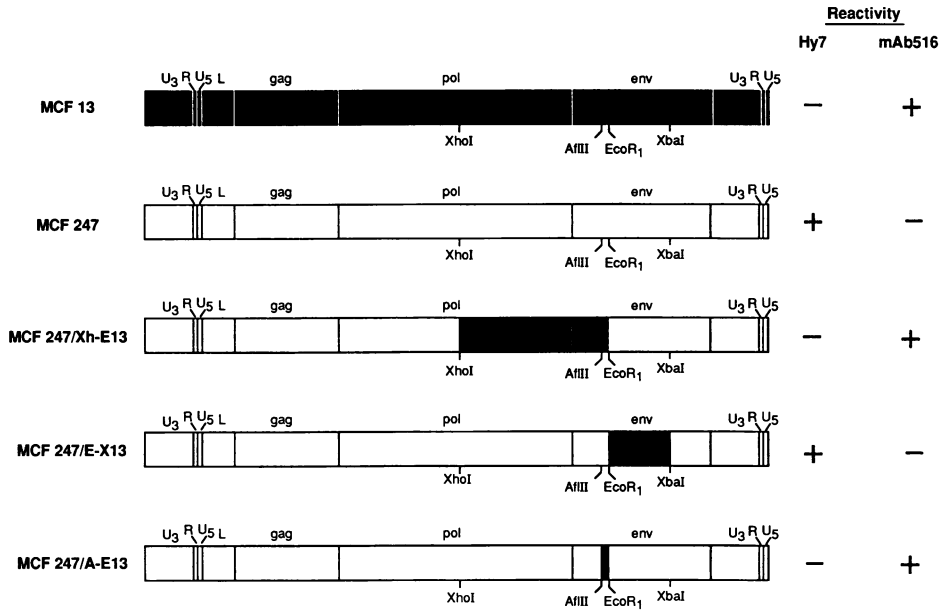

FIG. 1. Reactivities of chimeric MuLVs constructed between MCF 247 and MCF 13. MCF 13, MCF 247, and the chimeric MuLV proviruses are represented by bar diagrams which indicate the locations of the three structural genes (*gag*, *pol*, and *env*), the leader sequence (L), and the elements of the long terminal repeat (U_3, R, and U_5). The chimeric MuLVs correspond to MCF 247 proviruses with fragments derived from MCF 13 (shaded regions) and were constructed as described in Materials and Methods. The Hy 7 and MAb 516 reactivities were ascertained by a membrane immunofluorescence assay on transfected mouse cell lines.

molecularly cloned proviruses, were originally obtained from E. M. Scolnick (Merck Sharp and Dohme Research Laboratories, West Point, Pa.). The derivation of FB-29, a molecular clone of the I-5 strain of F-MuLV, has been previously described (46). The mouse strain utilized in this study was NFS/N.

MAbs. The derivations and specificities of MAbs 514 and 516 (9) and of Hy 7 (12) have been previously described. MAb 573, which is broadly reactive with MuLVs, was generously supplied by Bruce Chesebro (Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Mont.).

Construction of chimeric plasmids. MCF 13 was cloned in a pUC8 plasmid at a unique *Eco*RI site in the *env* gene (unpublished data). MCF 247, cloned at the *Pst*I site in pBR322, was recloned in another plasmid to facilitate the subsequent manipulations. The polylinker region of the plasmid pBSKS$^+$ (Stratagene) was excised by *Kpn*I and *Sac*I digestion. The plasmid was blunt ended with T4 DNA polymerase, and a *Pst*I linker (5′ GGCTGCAGCC 3′) was inserted by blunt-end ligation to generate a plasmid containing a unique *Pst*I site (pBSP$^+$) but no additional sites originally contained in the polylinker. The MCF 247 provirus, excised by *Pst*I digestion from the pBR322 plasmid, was then recloned into pBSP$^+$ (pBSP$^+$/MCF 247). To simplify the construction and analyses of chimeras, a 3.2-kb restriction enzyme fragment between a unique *Xho*I site in the *pol* gene and a unique *Xba*I site in the 3′ end of the *env* gene of MCF 247 was subcloned into pBSIIKS$^+$ (Stratagene). Constructions were done between this *Xho*I-*Xba*I MCF 247 subclone and fragments of the MCF 13 provirus. The chimeric *Xho*I-*Xba*I fragments were then inserted into a derivative of pBSP$^+$/MCF 247 (delta pBSP$^+$/MCF 247) in which the *Xho*I-*Xba*I fragment had been replaced with a conversion adaptor (5′ TCGAGCGCGCCT 3′ and 5′ CTAGAGGCGCGC 3′). The resulting chimeric proviruses were used to transfect NIH 3T3 or *M. dunni* cell lines. The chimeric MuLVs utilized in this study are presented in Fig. 1.

In vitro mutagenesis. Mutations were introduced into plasmids (pBSIIKS$^+$) containing the *Xho*I-*Eco*RI fragment of MCF 247 or MCF 13 (which includes the amino-terminal coding region of the SU protein) by use of a mutagenesis kit (200510; Stratagene) according to the manufacturer's instructions. The procedure utilizes a unique *Sca*I site present in pBSIIKS$^+$ and initiation primers complementary to that site. The *Xho*I-*Eco*RI subclones were utilized for the generation of mutations in order to exclude a *Sca*I site present in the carboxy-terminal coding region of the SU protein. After mutagenesis, the fragments were purified and ligated in a plasmid (pBSIIKS$^+$) containing the *Eco*RI-*Xba*I fragment of MCF 247 to obtain mutated *Xho*I-*Xba*I fragments. These fragments were subsequently used to generate mutated proviruses by insertion into delta pBSP$^+$/MCF 247.

Transfection of murine cells. Transfection of NIH 3T3 or *M. dunni* cells was performed by the calcium phosphate precipitation procedure with a mammalian transfection kit (200285; Stratagene). The proviruses were excised from the plasmid by cleavage with the appropriate restriction endonuclease (*Eco*RI for pUC8/MCF 13 and *Pst*I for pBSP$^+$/MCF 247 and derivatives) before transfection. Each transfection utilized 20 to 30 μg of plasmid DNA.

Focal immunoassay and membrane immunofluorescence. A focal immunofluorescence assay was used to evaluate the transfections and to detect ecotropic or polytropic MuLVs from infected mice. The focal immunofluorescence assay was performed on monolayers of live *M. dunni*, SC-1, NIH 3T3, or mink CCL-64 cells as previously described (45). MuLVs from infected NFS/N mice were detected in parallel infectious center (IC) assays of spleen and thymus cells 5 to 8 weeks after neonatal infection by use of MAb 514, MAb 516, or Hy 7 on mink CCL-64 cells and mouse SC-1 cells. Transfection of NIH

TABLE 1. Two mutually exclusive epitopes of polytropic MuLVs

Virus (reference)	Parent ecotropic MuLV	Mouse strain origin	Reactivity[a]		
			MAb 514	Hy 7	MAb 516
Viruses with an endogenous ecotropic parent					
Mo-MCF (5)[b]	Mo-MuLV	BALB/Mo	+	+	−
MCF 247 (27)	Akv	AKR	+	+	−
AKR 6AS (8)	Akv	AKR	+	+	−
AKR 6AT (8)	Akv	AKR	+	+	−
AKR L3 (13)	Akv	AKR	+	+	−
M60P-S (22)	Akv	AKR	+	+	−
M62P-S (22)	Akv	AKR	+	+	−
M72P-S (22)	Akv	AKR	+	+	−
M73P-S (22)	Akv	AKR	+	+	−
M75P-T (22)	Akv	AKR	+	+	−
M75P-S (22)	Akv	AKR	+	+	−
M81P-S (22)	Akv	AKR	+	+	−
Akv-1-C36 (13)	Akv-1	NFS congenic	+	+	−
Akv-2-C34 (13)	Akv-2	NFS congenic	+	+	−
C58L1B (13)	C58v	C58	+	+	−
MCF 13 (13)	Akv	AKR	+	−	+
AKR L4 (13)	Akv	AKR	+	−	+
AKR L5 (13)	Akv	AKR	+	−	+
Akv-1-C44-1 (34)	Akv-1	NFS congenic	+	−	+
Akv-1-C44-2 (34)	Akv-1	NFS congenic	+	−	+
Viruses with an exogenous ecotropic parent					
368-2S (19)	F-MuLV	NFS/N	+	+	−
368-3T (19)	F-MuLV	NFS/N	+	+	−
368-5T (19)	F-MuLV	NFS/N	+	+	−
F-MCF-18D (6)	F-MuLV	BALB/c	+	−	+
F-MCF-1 (31)	F-MuLV	NIH Swiss	+	−	+
F-MCF-1E (10)	F-MuLV	NIH Swiss	+	−	+
MCF-FrNx (1)	F-MuLV	NFS/N	+	−	+
383-1T (20)	Mo-MuLV	NFS/N	+	−	+
383-1S (20)	Mo-MuLV	NFS/N	+	−	+
383-2T (20)	Mo-MuLV	NFS/N	+	−	+
383-4T (20)	Mo-MuLV	NFS/N	+	−	+
383-4S (20)	Mo-MuLV	NFS/N	+	−	+
383-5T (20)	Mo-MuLV	NFS/N	+	−	+
383-5S (20)	Mo-MuLV	NFS/N	+	−	+
HIX (24)	Mo-MuLV	Unknown	+	−	+
V33 (48)	In vitro construct	HRS/J	+	−	+
368-2T (19)	F-MuLV	NFS/N	+	−	−
368-5S (19)	F-MuLV	NFS/N	+	−	−
368-6T (19)	F-MuLV	NFS/N	+	−	−
368-7T (19)	F-MuLV	NFS/N	+	−	−
368-7S (19)	F-MuLV	NFS/N	+	−	−

[a] Membrane immunofluorescence assays of mouse cell lines chronically infected with the designated polytropic MuLV isolate. Polytropic MuLVs reactive with Hy 7 or MAb 516 are boxed to clearly illustrate that the reactivities are mutually exclusive.

[b] Mo-MCF is a polytropic MuLV isolated from a BALB/c mouse strain with an endogenous Mo-MuLV (BALB/Mo).

3T3 or *M. dunni* cells was monitored by the focal immunofluorescence assay with MAb 573 until the cells were more than 90% fluorescent, indicating nearly complete infection. The infected cells were then trypsinized, and cell suspensions were assayed for reactivity with the polytropic-specific MAb 514 or 516 or Hy 7 by a membrane immunofluorescence assay as previously described (12).

DNA sequencing and oligonucleotide systhesis. DNAs were sequenced by the dideoxy-chain termination method (Gem Seq K/RT sequencing system; Promega Biotec, Madison, Wis.). Oligonucleotides were synthesized with an Applied Systems 380B DNA synthesizer (Foster City, Calif.).

RESULTS

Antigenic subclasses of polytropic MuLVs defined by reactivity with MAb 516 and Hy 7. Many polytropic MuLVs, isolated in this and in other laboratories, were examined for their reactivities to MAb 516 and Hy 7 (Table 1). These included polytropic MuLVs derived from different mouse strains and from different exogenous or endogenous ecotropic parents and, in one case (V33), an in vitro construct with an SU-coding sequence from a genomic library. Nearly all polytropic MuLVs reacted with either MAb 516 or Hy 7; however, none of the isolates from any source were found to react with both of the MAbs. MAb 514 reacted with all polytropic isolates examined, which confirmed and extended previous results (9). A group of polytropic MuLVs were reactive with MAb 514, but not with either Hy 7 or MAb 516 (Table 1, 368-2T, 5S, 6T, 7T, and 7S). These viruses were isolated from F-MuLV$_{57}$-infected NFS/N mice after extensive selection on mink cells (20). In experiments described below, these exceptional polytropic MuLVs constituted a very minor proportion of recombinants

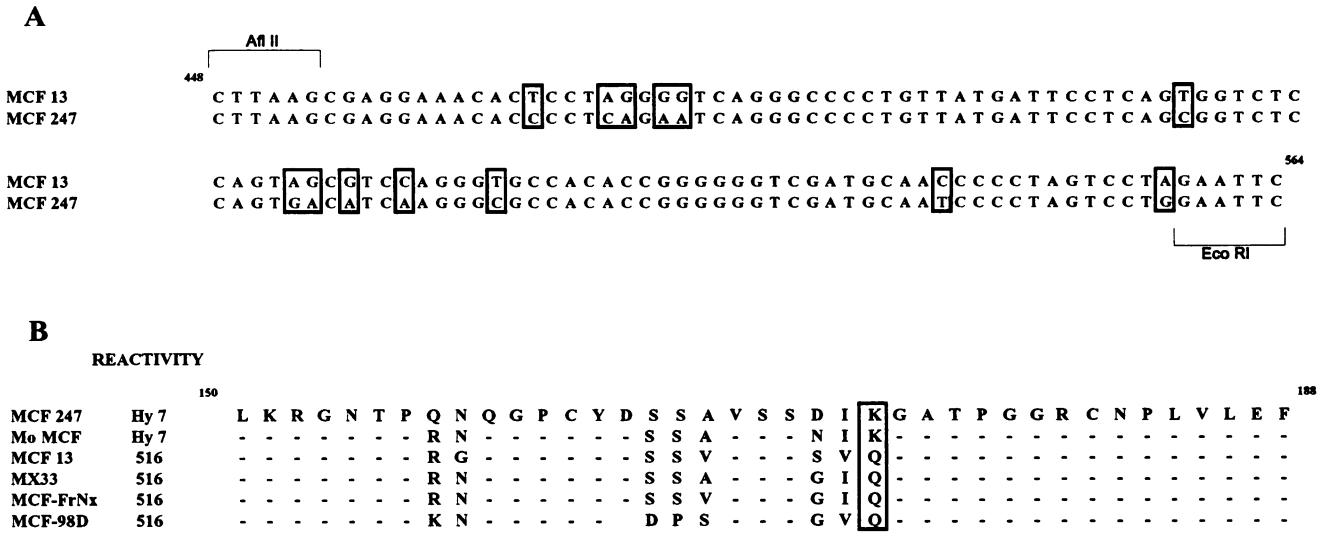

FIG. 2. Nucleotide and amino acid sequence comparisons of polytropic MuLVs. (A) The nucleotide sequence from an *Afl*II site (nucleotide 448) to an *Eco*RI site (nucleotide 564) of the *env* gene of MCF 13 was determined as described in Materials and Methods and aligned with the published sequence of MCF 247 (30). The locations of positions in the alignment which differ are enclosed in borders. (B) Amino acid sequences were deduced from the nucleotide sequences from the *Afl*II to the *Eco*RI sites in the SU-encoding region of the *env* genes of the MuLVs. The nucleotide sequence from *Afl*II to *Eco*RI of MCF-98D was kindly supplied by B. Chesebro (8a). The sequences of this region of MCF 247, Mo-MCF, MX33, and MCF-FrNx polytropic MuLVs have been reported elsewhere (1, 5, 30, 48). Dashes indicate amino acid identity at the given position in all the sequences. The single position at which the amino acid divergence exhibited complete consensus with reactivity to Hy 7 or MAb 516 is boxed. Hy 7 and MAb 516 (516) reactivities were determined by immunofluorescence on chronically infected mouse cell lines.

detected in NFS/N mice. Our results suggest that the reactivities to MAb 516 and Hy 7 are mutually exclusive and that the reactive epitopes correspond to alternate allelic structures.

Localization of sequences encoding MAb 516 or Hy 7 reactivity. The envelope protein complex appears important for reactivity with MAb 516 and Hy 7 in that both are reactive with the gp70-p15E complex in radioimmune precipitation and immunoblots but not with gp70 alone (9, 12). Thus, it is not entirely clear whether the antigenic subclasses are the result of differences encoded by the polytropic region of the *env* gene or of differences in other regions. The observation that the same ecotropic MuLV can give rise to both antigenic subclasses (e.g., MCF 247 and MCF 13 [Table 1]) suggests that the nonecotropic region of the SU protein is very important for the reactivity to the two MAbs. However, it is possible that differences in the points of recombination could generate the dichotomy. To determine if the MAb 516 and Hy 7 reactivities are dependent on the identities of the polytropic sequences in the *env* gene, chimeric viruses were constructed between two molecularly cloned viruses, MCF 247 and MCF 13, which differed in their reactivities to MAb 516 and Hy 7 (Fig. 1). MCF 247 reacted with Hy 7, and MCF 13 reacted with MAb 516. Both viruses were derived from the same parental ecotropic virus (Akv), which facilitated the construction of recombinant plasmids. The reactivities of the constructed viruses were examined by membrane immunofluorescence on infected cells after DNA transfection. Analyses of two chimeras, MCF 247/Xh-E13 and MCF 247/E-X13, identified sequences in the 5′ region of the *env* gene which influenced MAb 516 or Hy 7 reactivity (Fig. 1). The 5′ SU-encoding sequences of MCF 247/Xh-E13 extending 564 bases to the *Eco*RI site were derived from MCF 13, while the remainder of the SU-encoding sequences were from MCF 247. Like MCF 13, this chimera reacted with MAb 516. Conversely, MCF 247/E-X13, which contained 5′ SU-encoding sequences from MCF 247 and 3′ SU-encoding sequences from MCF 13, was, like MCF 247, reactive with Hy 7. Sequences determining reactivities to MAb 516 and Hy 7 were localized to a much smaller region by analyses of the MCF 247/A-E13 chimera. This virus was identical to MCF 247, except for a 117-bp sequence of MCF 13 extending from an *Afl*II site to the *Eco*RI site, and reacted with MAb 516 similarly to MCF 13.

Sequence comparisons of Hy 7- and MAb 516-reactive polytropic MuLVs and the modification of Hy 7 and MAb 516 reactivities by point mutation. The results above indicate that Hy 7 and MAb 516 reactivities are influenced by sequences between the *Afl*II and *Eco*RI sites of the polytropic *env* gene (nucleotides 448 to 564 of the SU-encoding region). The nucleotide sequence of MCF 13 was determined in this region and differed by 13 nucleotides from the published sequence of MCF 247 (Fig. 2A). The deduced amino acid sequence encoded between the *Afl*II and *Eco*RI sites of MCF 13 was compared with that of MCF 247 as well as those of other polytropic MuLVs which were characterized with respect to their reactivities to Hy 7 or MAb 516 (Fig. 2B; Table 1). We found eight positions which exhibited amino acid divergence among the polytropic MuLVs in this region. Only one of the eight positions in this region exhibited consensus with respect to Hy 7 or MAb 516 reactivity. Both Hy 7-reactive viruses contained lysine at position 173, while all four MAb 516-reactive MuLVs contained glutamine.

Point mutations were introduced to change the amino acid at position 173 of the SU protein of MCF 247/Xh-E13 (MAb 516 reactive) from glutamine to lysine and that of MCF 247 (Hy 7 reactive) from lysine to glutamine (Fig. 3). In both cases, alteration of the codon was effected by a single base change. Mutated viruses were analyzed after transfection to determine if this residue influenced reactivity to the MAbs. The results of these analyses (Fig. 3) indicated that the identity of this amino acid (glutamine or lysine) in MCF 247/Xh-E13 determined

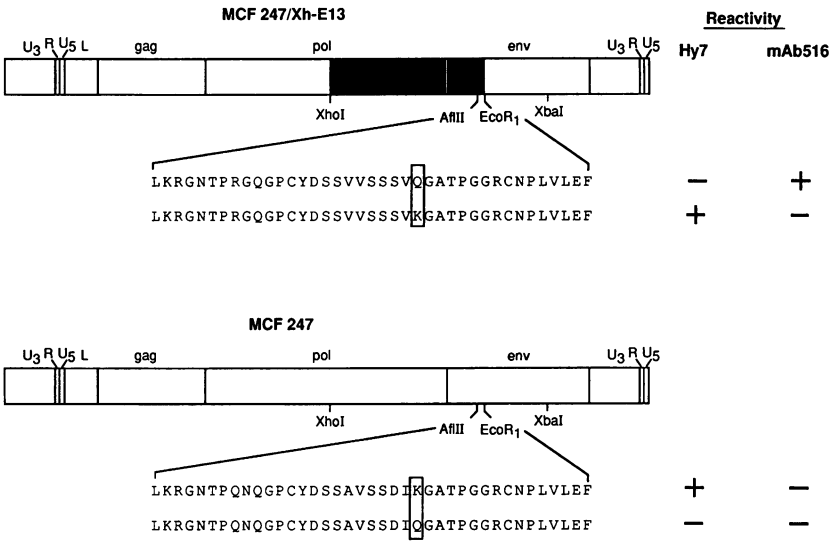

FIG. 3. Alteration of the SU protein sequences of MAb 516- and Hy 7-reactive polytropic MuLVs. A mutation (C to A) was introduced at nucleotide 517 of the *env* gene of MCF 247/Xh-E13 to effect a glutamine-to-lysine coding change. The mutagenesis was accomplished with a mutagenic primer (5′ GTCTCCAGTAGCGTCAAGGGTGCCACACCGG 3′). A mutation (A to C) was introduced at the same position in MCF 247 with a mutagenic primer (5′ GGTCTCCAGTGACATCCAGGGCGCCACACCGGG 3′) to effect a lysine-to-glutamine coding change. The alterations in the amino acid sequences are boxed. Mouse cell lines were transfected by the mutated proviruses, and the Hy 7 and MAb 516 reactivities were determined in the experiments presented in Fig. 4 and 5. Shading and abbreviations are as for Fig. 1.

reactivity to MAb 516 or Hy 7. Changing the glutamine to lysine in this chimeric MuLV resulted in a conversion from a MAb 516-reactive MuLV to one which was reactive with Hy 7 (Fig. 4). Similarly, changing the lysine to glutamine at position 173 of MCF 247 abolished reactivity to Hy 7, suggesting that lysine at this position is necessary for reactivity to that antibody. However, with MCF 247 the amino acid conversion did not result in a conversion of the reactivity to MAb 516 (Fig. 5). This result suggests that glutamine at position 173 is not sufficient for reactivity to MAb 516 and that additional difference(s) between MCF 247 and MCF 13 also influence reactivity to this MAb.

Our analyses indicate that the two antigenic subclasses of polytropic MuLVs are determined by alternative allelic nucleotide sequences and very likely are the result of recombination with distinct sets of endogenous polytropic *env* genes.

Detection of polytropic MuLVs from ecotropic MuLV-infected mice: distinct distributions of polytropic MuLV antigenic subclasses are expressed after infection with different ecotropic MuLVs. The results presented in Table 1 suggest that the frequencies of occurrence of the antigenic subclasses of polytropic MuLVs may differ in the same mouse strain after inoculation with different ecotropic viruses. All polytropic isolates from NFS/N mice inoculated with Mo-MuLV were reactive with MAb 516, whereas polytropic isolates from NFS/N mice inoculated with F-MuLV were reactive with Hy 7 or unreactive with either MAb. Protocols for the isolation of polytropic MuLVs have generally included extensive ex vivo selection on mink cells (4, 19, 24, 27). However, in more recent studies we observed that polytropic MuLVs generated in AKR/J mice, which have an endogenous ecotropic MuLV, were much more efficiently detected on mouse cell lines (18, 22). In the present study, we compared the efficiency of detection of polytropic MuLVs on mouse and mink cell lines after inoculation of NFS/N mice with F-MuLV$_{57}$ or Mo-MuLV, to determine which system would minimize the bias imposed by ex vivo selection. We found the efficiency of detection of polytropic MuLVs with the mouse cell line to be vastly greater than that with mink cells (Table 2). Polytropic MuLVs from the spleens of Mo-MuLV-infected mice were detected 10,000-fold more efficiently on mouse cells than on mink cells, and those from the thymus were detected 100-fold more efficiently. No polytropic isolates were detected in IC assays with mink cells using up to 10^6 spleen or thymus cells from F-MuLV$_{57}$-infected mice. Assays using the mouse cell line detected substantial levels of polytropic MuLVs from the spleens and thymuses of mice infected with Mo-MuLV, F-MuLV$_{57}$, or a third ecotropic MuLV, FB-29 (Table 2). The results presented in Table 2 correspond to the sum of parallel IC assays using MAb 516 or Hy 7. These values were corroborated in parallel assays using MAb 514 (data not shown). MAb 514 was reactive with all polytropic MuLVs including those isolates which were not reactive with either MAb 516 or Hy 7 (Table 1). Thus, polytropic MuLVs corresponding to the two antigenic subclasses constituted the major proportion of polytropic MuLVs detected from infected mice.

A comparison of the antigenic subclasses of polytropic MuLVs detected in the experiments described above (Table 2) is presented in Fig. 6. The data are expressed as the percentage of foci detected, with the combined total of MAb 516 and Hy 7 foci as 100%. Results obtained with each mouse in the analyses are presented to illustrate the variability observed among individual mice. Most Mo-MuLV-infected mice expressed both subclasses of polytropic MuLVs in the spleen and the thymus, although the percentage of MAb 516-reactive MuLVs usually exceeded that of the Hy 7-reactive MuLVs (Fig. 6A and B). F-MuLV$_{57}$-infected mice exhibited a strikingly different pattern of polytropic MuLV expression (Fig. 6C and D). High percentages of Hy 7-reactive MuLVs were detected in both the spleen and the thymus, and most mice exhibited no MAb 516-reactive MuLVs. Mice infected with a second F-MuLV strain, FB-29, expressed both subclasses of polytropic MuLVs in the spleen and thymus (Fig. 6E and F). This result was similar to that obtained with Mo-MuLV-

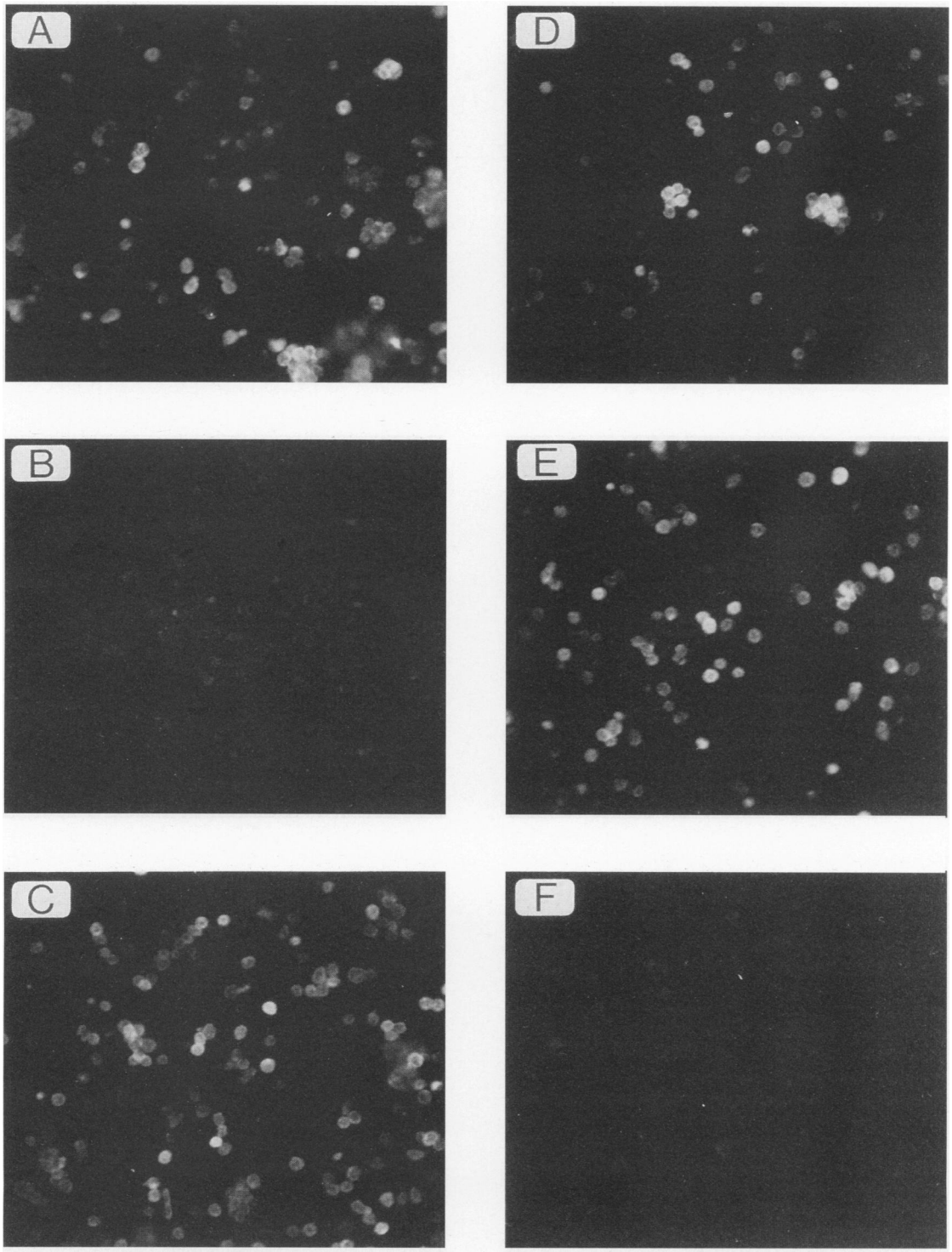

FIG. 4. Conversion of MAb 516 reactivity to Hy 7 reactivity by point mutation. *M. dunni* cells transfected by the chimeric virus MCF 247/Xh-E13 were assayed by membrane immunofluorescence for reactivity to MAb 573 (A) to confirm infection of the cells.The antigenic subclass of the virus was determined by reactivity to Hy 7 (B) or MAb 516 (C). The derivative of MCF 247/Xh-E13 in which the glutamine at position 173 of the SU protein was changed to a lysine by in vitro mutagenesis (see Fig. 3) was also tested for reactivity with MAb 573 (D), Hy 7 (E), or MAb 516 (F).

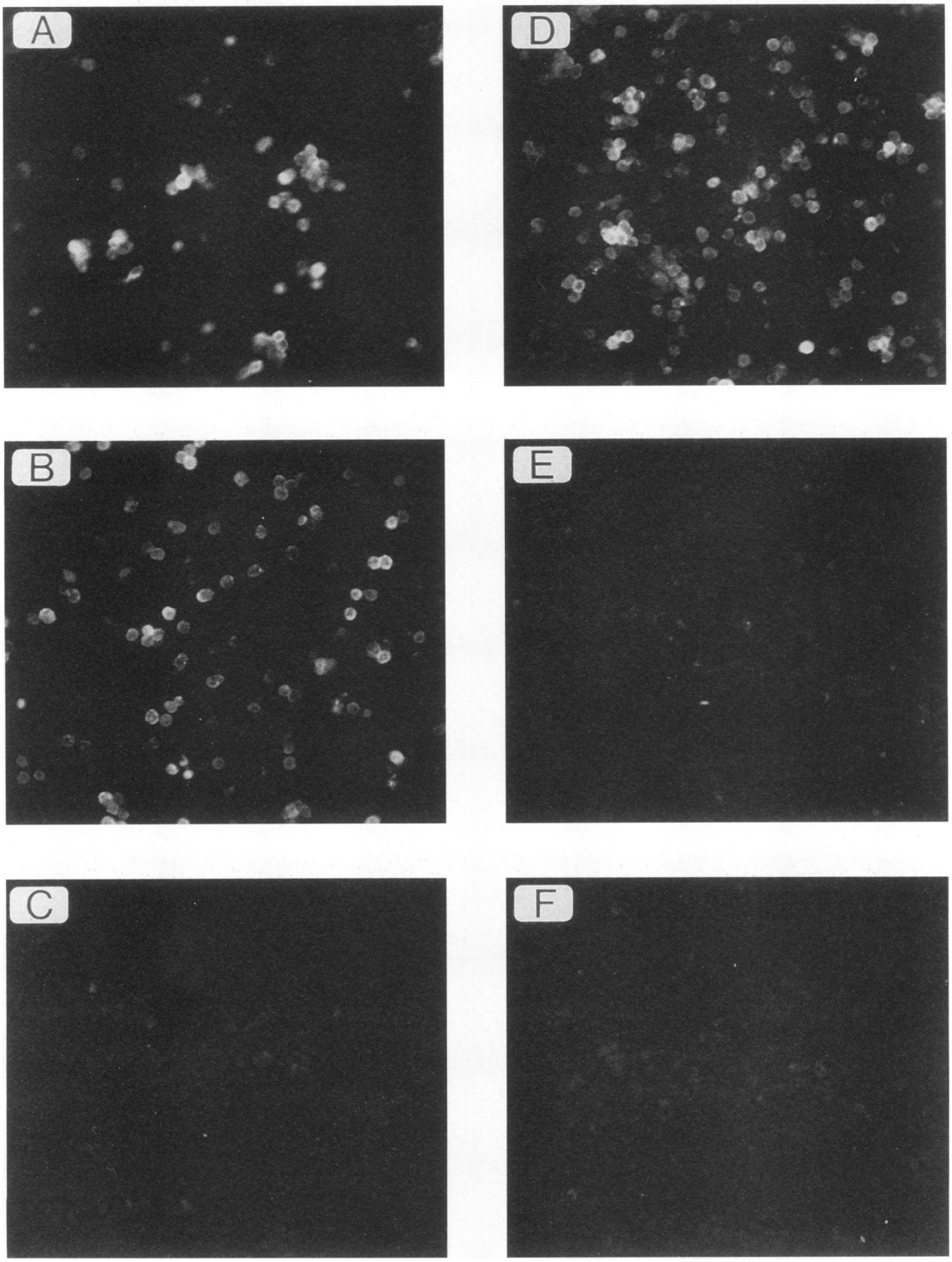

FIG. 5. Alteration of Hy 7 reactivity by point mutation. Membrane immunofluorescence assays were performed on *M. dunni* cells transfected by MCF 247 with MAb 573 (A) to confirm infection of the cells. The antigenic subclass of the virus was determined by reactivity to Hy 7 (B) or MAb 516 (C). Parallel assays of a mutant of MCF 247 in which the lysine at position 173 of the SU protein was changed to a glutamine by in vitro mutagenesis (Fig. 3) determined reactivities with MAb 573 (D), Hy 7 (E), and MAb 516 (F).

TABLE 2. IC titers of polytropic MuLVs from spleens or thymuses of infected NFS/N mice

Ecotropic MuLV[a]	No. of mice	Log IC titer (mean ± SD)[b] for cells of indicated type			
		Mouse (SC-1)		Mink (CCL-64)	
		Spleen	Thymus	Spleen	Thymus
Mo-MuLV	9	4.05 ± 0.67	3.95 ± 0.62	<1	1.96 ± 0.93
F-MuLV$_{57}$	8	4.05 ± 0.32	2.11 ± 0.63	<0	<0
FB-29	5	3.46 ± 0.27	2.92 ± 0.27	ND[c]	ND

[a] NFS/N mice were inoculated neonatally with Mo-MuLV, F-MuLV$_{57}$, or FB-29 and assayed at 5 to 8 weeks of age.
[b] The polytropic MuLV titer was determined as a sum of Hy 7-reactive and MAb 516-reactive foci scored by the focal immunofluorescence assay as described in Materials and Methods. The IC titer is the number of cells per million which scored as fluorescent foci in the assay. The titer is expressed as the average of the log of the ICs determined from individual mice.
[c] ND, not determined.

infected mice; however, the percentage of Hy 7-reactive MuLVs appeared higher in the spleens of FB-29-infected mice (Fig. 6E) than in mice infected with Mo-MuLV (Fig. 6A).

DISCUSSION

In this study, we have identified two major antigenic subclasses of polytropic MuLVs on the basis of their reactivity to MAb 516 or Hy 7. Nearly all polytropic isolates reacted with either MAb 516 or Hy 7, while no virus reacted with both MAbs, suggesting that they recognize mutually exclusive epitopes. Analyses of chimeric viruses and point mutagenesis studies mapped sequences necessary for the reactivity of the MAbs to the same codon in the polytropic region of the SU protein-encoding sequences. We have demonstrated the conversion of one subclass to the other by a single base change; thus, it is conceivable that mutation subsequent to recombination could contribute to the dichotomy. This seems unlikely, given that many mice exhibit approximately equal levels of both subclasses and the isolates appear stable upon ex vivo passage. We conclude that the two subclasses are the result of recombination of ecotropic MuLVs with two distinct subclasses of endogenous polytropic *env* genes.

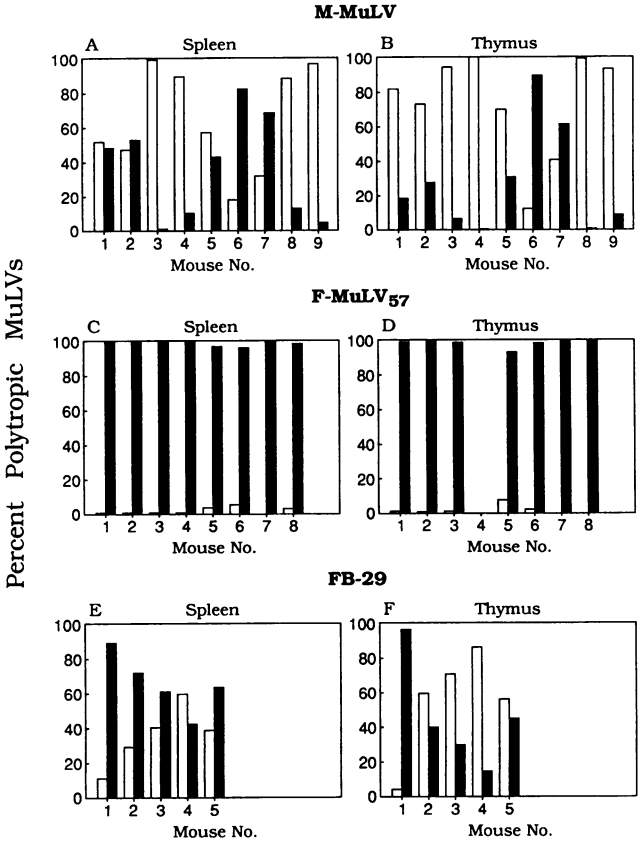

FIG. 6. Percentage of Hy 7- and MAb 516-reactive polytropic MuLVs detected from spleens or thymuses of infected mice. The assays presented in Table 2 are expressed as the percentages of Hy 7- and MAb 516-positive foci which were scored for individual mice. Results were obtained with spleen and thymus cells of mice infected with Mo-MuLV (nine mice) (A and B), F-MuLV$_{57}$ (eight mice) (C and D), or FB-29 (five mice) (E and F). Open bars, MAb 516-reactive MuLVs; closed bars, Hy 7-reactive MuLVs.

Stoye and Coffin have also identified two subclasses of endogenous polytropic *env* genes by direct analyses of genomic DNA (48, 49). It is clear that the subclasses described by Stoye and Coffin do not correspond to the antigenic subclasses defined by MAb 516 and Hy 7. The sequences they have characterized, termed polytropic and modified polytropic, are distinguished by a small in-frame deletion near the middle of the SU protein-encoding sequences, approximately 250 bp from the codon which influences reactivities to Hy 7 and MAb 516. Furthermore, both Hy 7-reactive and MAb 516-reactive MuLVs are found among polytropic isolates which do not contain a deletion. Modified polytropic sequences constitute about 30% of the endogenous polytropic sequences in several mouse strains, yet only one naturally occurring isolate containing such a sequence has been described (31). This suggests that modified polytropic sequences do not frequently participate in recombination to generate polytropic MuLVs. Our analyses of polytropic MuLV isolates (Table 1), as well as the analyses of populations of polytropic MuLVs released from tissues of infected mice (Fig. 6), indicate that Hy 7-reactive and MAb 516-reactive polytropic MuLVs are frequently generated and, together, constitute a major proportion of the polytropic viruses detected. We are currently investigating the distribution of endogenous polytropic sequences that encode SU proteins reactive with Hy 7 or MAb 516.

It is possible that reactivity to MAb 516 or Hy 7 could signal different infectivity properties of polytropic MuLVs. The receptor-binding domain of the SU protein contains two variable regions, termed VRA and VRB (2). VRA is a strong determinant of receptor choice, while VRB influences the stability of receptor binding. The amino acid residue which altered MAb 516 and Hy 7 reactivities lies within VRB and could influence receptor binding which could, in turn, affect infectivity.

Most protocols for the analysis and isolation of polytropic MuLVs involve passage and limiting dilution of the viruses in

mink cells (4, 19, 24, 27). We found that polytropic MuLVs were detected much more efficiently on mouse cells than on mink cells (Table 2). It seems likely that many of the polytropic MuLVs which have been isolated and studied may not be representative of the prevalent recombinant polytropic MuLVs which arise in mice. Earlier studies, in which polytropic MuLVs from Mo-MuLV-infected NFS/N mice were isolated by endpoint dilution on mink cells, detected only MAb 516-reactive MuLVs (20). In the present study, we have identified major polytropic MuLV populations of both antigenic subclasses from Mo-MuLV-infected NFS/N mice (Fig. 6A and B). It is noteworthy that the ratio of MAb 516-reactive to Hy 7-reactive MuLVs from thymus cells of Mo-MuLV-infected mice was approximately threefold higher on mink cells than on mouse cells (data not shown), indicating a selection for MAb 516-reactive MuLVs in this system. Although polytropic MuLV isolates which are much more infectious for mouse cells than mink cells have been described, (18, 22), it is unlikely that in vitro tropism of the viruses accounts for the huge difference between the detection of polytropic MuLVs on mouse cells and that on mink cells. Viral pseudotyping of the polytropic MuLV RNA in ecotropic virions could account for the discrepancy. The observation that polytropic MuLVs from the thymuses of Mo-MuLV-infected mice can be detected on mink cells whereas polytropic MuLVs from the spleens of Mo-MuLV-infected mice are rarely detected on mink cells (Table 2) suggests that the extent of pseudotyping may differ in different tissues.

Our findings indicate that infection of NFS/N mice with different ecotropic MuLVs gives rise to distinct populations of polytropic MuLVs. There are a number of possible explanations for this observation. If recombination occurs during reverse transcription of heterodimeric RNA (33), ecotropic MuLVs could exhibit specificity at the level of heterodimer formation with different endogenous RNA transcripts. Ecotropic MuLVs differ in their tissue and cell tropisms (14, 19, 23, 35, 41); thus, the type of recombinant virus generated could reflect the type of RNA transcripts available for heterodimer formation in a particular cell type. It is also conceivable that polytropic MuLVs are generated by a mechanism involving DNA. If so, homology between the ecotropic and endogenous retroviral sequences could influence the recombination frequency. Alternatively, differences in populations of polytropic MuLVs observed after inoculation of different ecotropic MuLVs could be the result of in vivo selection of the recombinant viruses subsequent to their generation.

Differences in polytropic MuLV populations observed after infection of mice with different ecotropic MuLVs may be relevant to pathology. Mo-MuLV and F-MuLV$_{57}$ cause lymphocytic leukemia and erythroleukemia, respectively, and exhibit strikingly different proportions of the polytropic MuLV subclasses (Fig. 6). The FB-29 strain of F-MuLV also differs from F-MuLV$_{57}$ with regard to polytropic MuLV subclasses; however, this does not preclude the possibility that F-MuLVs generate a particular polytropic species involved in the induction of erythroleukemia. A high proportion of thymomas induced by Mo-MuLV exhibit integration of a polytropic MuLV provirus near a c-*onc* gene (47, 51). Both subclasses of polytropic MuLVs were identified in most Mo-MuLV-infected mice (Fig. 6A and B). Preliminary studies suggest that MAb 516-reactive and Hy 7-reactive MuLV isolates from these mice exhibit different infectious properties in vitro. Given that such differences might reflect differences in infectivity for particular cell types in vivo, it is possible that a particular polytropic MuLV subclass is involved in the aberrant expression of c-*onc* genes in Mo-MuLV-induced tumors.

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