Friend and Moloney murine leukemia viruses specifically recombine with different endogenous retroviral sequences to generate mink cell focus-forming viruses

(RNase T1-resistant oligonucleotides/genome structure/antigenic heterogeneity/monoclonal antibodies)

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ABSTRACT A group of mink cell focus-forming (MCF) viruses was derived by inoculation of NFS/N mice with Moloney murine leukemia virus (Mo-MuLV 1387) and was compared to a similarly derived group of MCF viruses from mice inoculated with Friend MuLV (Fr-MuLV 57). Antigenic analyses using monoclonal antibodies specific for MCF virus and xenotropic MuLV envelope proteins and genomic structural analyses by RNase T1-resistant oligonucleotide fingerprinting indicated that the Moloney and Friend MCF viruses arose by recombination of the respective ecotropic MuLVs with different endogenous retrovirus sequences of NFS mice.

Friend and Moloney ecotropic murine leukemia viruses (Fr-MuLV and Mo-MuLV, respectively) induce hematopoietic proliferative diseases when inoculated neonatally into susceptible strains of mice. Fr-MuLV induces primarily erythroleukemia, whereas Mo-MuLV induces lymphocytic leukemia (1–4). Mink cell focus-forming (MCF) viruses, which are recombinants between the inoculated ecotropic virus and virus sequences endogenous to the mouse, can be isolated from animals infected with either Fr-MuLV or Mo-MuLV in both the preleukemic and leukemic stages of disease and have been suggested to be involved in the disease process (4–7). In an attempt to identify the nonecotropic viral sequences in mice that give rise to MCF viruses, we have recently reported detailed analyses of MCF viruses derived from NFS/N mice inoculated with Fr-MuLV 57 (8). Two types of Friend MCF (Fr-MCF) viruses were isolated that correspond to recombination of Fr-MuLV 57 with distinct but related NFS endogenous retroviral sequences. These sequences differed from sequences found in MCF viruses derived from NFS mice congenic for the endogenous ecotropic virus genes of AKR mice (NFS.AKv-1 and NFS.AKv-2), suggesting that different ecotropic viruses may selectively recombine with different endogenous retroviral sequences to generate MCF viruses.

In the present report, we have examined a family of MCF viruses derived from NFS/N mice after inoculation of Mo-MuLV in order to compare them with FR-MCF viruses.

MATERIALS AND METHODS

Cells, Viruses, and Mice. MCF viruses were isolated from NFS/N mice inoculated neonatally with Mo-MuLV 1387 (9) by procedures that have been described (8). Each virus isolate in these analyses was cloned by several cycles of infection near the limiting dilution and was evaluated for homogeneity on the basis of RNase T1-resistant oligonucleotide fingerprinting. Cloned MCF viruses were propagated for further analyses on a Mus dunni cell line originated by Chattapadhyay et al. (10).

Assays. Focus assays of viruses on mink or SC-1 cells have been described (8, 11). The antigenic reactivities of MCF viruses with hybridoma antibodies were determined by the reaction of the antibodies with cell-surface antigens of virus-infected Mus dunni cells as measured by a previously described immunofluorescence assay (12).

RNase T1-Resistant Oligonucleotide Analyses. RNase T1-oligomerucleotide fingerprinting and mapping procedures and secondary analysis of T1-resistant oligonucleotides by identification of RNase A digestion products have been described (8).

RESULTS

Biological Properties of Mo-MCF Viruses. Mo-MCF viruses were isolated from the thymus and spleen of animals in the preleukemic and leukemic stages of disease (Table 1). All of the isolates induced cytopathic foci on mink lung fibroblasts (ATTC CCL64) and were infectious for both mink and mouse (SC-1) cells. Previous studies of Fr-MCF viruses derived from NFS mice indicated that one group of them (group I Fr-MCF viruses) were equally infectious for SC-1 and mink cells, whereas a second group (group II) were 1000–10,000-fold more infectious for mink cells than for SC-1 cells (8). All Mo-MCF isolates examined in this study exhibited approximately equal infectivities for mink and mouse cells and, thus, resembled in this respect group I Fr-MCF viruses (data not shown).

Mo-MCF viruses have been reported to be directly oncogenic when inoculated neonatally into susceptible mouse strains (4, 5). Although our data are not yet complete for all of the Mo-MCF viruses in this study, we induced thymic lymphomas in NFS and AKR mice with some of the isolates. This oncogenicity is in contrast to the activity of either group I or group II Fr-MCF viruses, neither of which induced disease in the absence of ecotropic MuLV (8).

Antigenic Analyses of Mo- and Fr-MCF Viruses. Several monoclonal antibodies have been described that react specifically with env antigens of recombinant MCF or with both MCF and xenotropic viruses but not with ecotropic Fr- or Mo-MuLV (12–14). We used these antibodies to compare the nonecotropic envelope antigenic determinants present in Mo- and Fr-MCFs. The Mo-MCF viruses were antigenically distinguishable from the Fr-MCF viruses (Table 1). In particular, Mo-MCF viruses could be distinguished from Fr-MCF viruses.

Abbreviations: MuLV, murine leukemia virus; Mo-MuLV, Moloney MuLV; Fr-MuLV, Friend MuLV; MCF virus, mink cell focus-forming virus; Fr- and Mo-MCF, Friend and Moloney MCF viruses.

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Table 1. Monoclonal antibody reactions with Fr- and Mo-MCF-infected Mus dunni cells

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*Virus designations ending in S or T indicate splenic or thymic origin, respectively.
†Antibodies 514 and 522 are specific for gp70. Antibodies Hy7, 502, 508, 513, and 522 are specific for complex of gp70 and p15E.
‡MCF viruses obtained from preleukemic mice 3–4 weeks after inoculation of ectopic Mo-MuLV.
§MCF viruses obtained from leukemic mice 10 weeks after inoculation of Mo-MuLV 1387 (Mo-MCF viruses) or 6 weeks after inoculation of Fr-MuLV 57 (Fr-MCF viruses).

MCF viruses by their reactivities to antibodies 508, 513, 516, and 522 and by their low reactivity with 502. Two groups of Fr-MCF viruses previously identified (group I and group II; Table 1) on the basis of their relative infectivities for SC-1 and mink cells and the specific endogenous NFS sequences they respectively contain (8) were distinguished by their reactivity to Hy7.

**Comparison of T1-Resistant Oligonucleotides of Fr- and Mo-MCF Viruses.** Isolates of both groups of Fr-MCF viruses contained extensive regions of substitution, including gag, pol, and env gene sequences (8). In Fig. 1 the endogenously derived oligonucleotides are identified in the RNase T1-resistant oligonucleotide fingerprints of representative isolates of both Fr-MCF groups.

Fig. 2 shows the oligonucleotide fingerprints of Mo-MuLV and of seven Mo-MCF isolates. The identity of the numbered oligonucleotides was confirmed in all viruses by analyses of their RNase A digestion products (data not shown). The Mo-MCF viruses share several endogenously derived oligonucleotides with Fr-MCF viruses (X7, X10, X11, X16, X21, and X31). In addition, 11 new oligonucleotides (X44–X54) that were not found in any Fr-MCF isolate were identified in the Mo-MCF viruses. Of these 11 oligonucleotides, 6 were identified in multiple Mo-MCF isolates (X44, X45, X46, X47, X48, and X53), suggesting that they were of endogenous origin. The remaining new oligonucleotides, which were identified only once in various isolates, may have arisen by point mutations subsequent to recombination and are not considered to be unambiguous markers of endogenous sequences.

**Oligonucleotide Maps of Fr- and Mo-MCF Viruses.** Fig. 3 shows the oligonucleotide maps of the two representative Fr-MCF viruses, Mo-MuLV, and the seven Mo-MCF isolates. Also included in Fig. 3 are bar diagrams depicting the regions of the viral genomes that are of endogenous and exogenous origin and regions that contain oligonucleotides unique to group I Fr-MCF, group II Fr-MCF, or Mo-MCF viruses. It is noted that we have included two Fr-MuLV 57 oligonucleotides (F10 and F49) as being of endogenous origin. These two oligonucleotides have been identified in substituted regions of MCF viruses other than Fr-MCF viruses (8). The precise location of many of the oligonucleotides indicated in Fig. 3 was deduced from published nucleotide sequences (15–22). Several oligonucleotides that were identified as endogenously derived oligonucleotides in the Fr-MCF viruses (X17, X22, X27, X34, and F49) are components of ectopic Mo-MuLV RNA (M39, M59, M52, M51, and M47, respectively). The occurrence of these oligonucleotides in endogenous NFS sequences implies some ambiguity on the assignment of regions of endogenous origin in the Mo-MCF genomes.

In contrast to the extensive substitutions found in Fr-MCF viruses, the endogenous sequences of the Mo-MCF viruses were limited to approximately the 5' half of the gp70-coding sequences, extending in some cases into the pol gene. Two additional features of the Mo-MCF endogenous sequences clearly distinguished them from the endogenous sequences of either MCF group derived from Fr-MuLV 57. (i) All Mo-MCF isolates contained oligonucleotide X45 (Fig. 3B), which maps within the substituted env sequences near oligonucleotides X21 and X7. We have identified an oligonucleotide that corresponds to X45 in the nucleotide se-
FIG. 2. RNase T1-resistant oligonucleotide fingerprints of Mo-MuLV and Mo-MCF viruses from NFS mice. $^{32}$P-labeled 70S virion RNA fingerprints were obtained and analyzed as in Fig. 1. Mo-MuLV oligonucleotides and Mo-MCF oligonucleotides identical to those of Mo-MuLV were prefixed by an M. Mo-MCF oligonucleotides not found in Mo-MuLV were prefixed by an X. Mo-MCF oligonucleotides prefixed by X that are also found in Fr-MCF viruses (Fig. 1) are numbered identically. The oligonucleotides encircled by broken lines are components of low molecular weight virion RNA from M. dunii cells and are not found in 35S poly A-containing RNAs. (A) Mo-MuLV propagated in FRE cells. (B–H) Mo-MCF viruses propagated in M. dunii cells: Mo-MCF 383-1S (B), Mo-MCF 383-1T (C), Mo-MCF 383-2T (D), Mo-MCF 383-4S (E), Mo-MCF 383-4T (F), Mo-MCF 383-5S (G), and Mo-MCF-383-5T (H).
Fig. 3. RNase T1-resistant oligonucleotide maps and bar diagrams representing 35S genomic RNAs of Fr-MCF viruses, Mo-MuLV, and Mo-MCF viruses. The order of T1-resistant oligonucleotides relative to the 3' terminus of each virus was approximated by obtaining fingerprints of 10–12 distinct size classes of polyadenylate-containing fragments of each virus as described (8). Precise positions of many oligonucleotides were deduced from the approximate map position and published nucleotide sequences (15-22) and are indicated by lines drawn from the oligonucleotide numbers to positions on the bar diagrams. Oligonucleotide numbers in parentheses juxtaposed to the Mo-MuLV map identify oligonucleotides found in Mo-MuLV that have been identified previously as endogenously derived oligonucleotides in Fr-MCF viruses. White...
sequence of an MCF derived from a different strain of Fr-MuLV (22). However, X45 was not present in any MCF derived from Fr-MuLV 57, indicating that different endogenous retroviral sequences participated in the generation of the Mo- and Fr-MCFs examined in this study. (ii) In two isolates (383-1S and 383-SS; Fig. 3B), the endogenously derived sequences extend into the 3' region of the pol gene and are signaled by the appearance of four new oligonucleotides (X44, X46, X47, and X48). These oligonucleotides are distinct from the oligonucleotides residing in the corresponding regions of either of the two Fr-MCF groups (Fig. 3A) and further support the contention that the Fr- and Mo-MCF viruses contain distinct endogenous sequences.

New oligonucleotides that occurred only once in our MCF isolates and others that were absent from only one isolate are likely to be the result of random mutations (23). Thus, our oligonucleotide data are consistent with the recombinant of Mo-MuLV with a single endogenous retrovirus sequence to generate MCF viruses. No consistent differences were found between Mo-MCF viruses derived from preleukemic or leukemic animals or between Mo-MCF viruses derived from the spleen and those from the thymus (Table 1).

DISCUSSION

In our studies thus far, we have identified three distinct endogenous sequences of NFS mice that participate in the generation of MCF viruses. Two of these were identified in MCF viruses derived from Fr-MuLV 57 and the other was identified in Mo-MCF viruses. It is not clear what the determining factors are for the apparent specificity of the sequestration of endogenous sequences in generation of MCF viruses. Several possibilities are nonexhaustive. Fr- or Mo-MuLVs may recombine with any number of endogenous sequences, but many of these may yield poorly infectious recombinant viruses, and these would not be detected in our analyses. However, the specificity we have observed does not appear to be the result of preferential selection of certain infectious MCF viruses among many during our isolation procedure because our isolation procedures are invariant and serological analyses of uncultured virus populations or leukemic cells from these mice reflect the respective MCF antigens only (unpublished observations). These results suggest limited heterogeneity of MCF viruses generated. If virion RNA heterodimers are involved in the recombinational process (24-27), the specificity could reflect preferential packaging of different endogenous retroviral transcripts in Fr- and Mo-MuLV virions. Alternatively, Fr- and Mo-MuLVs could infect different cell types in which different endogenous retroviral sequences are transcribed. Finally, if recombination occurs in genomic DNA, the specificity could reflect preferential integration sites of Fr- and Mo-MuLVs.

It should be stressed that the oligonucleotide differences between Fr- and Mo-MCF env genes, although quite reproducible, are very slight. Fr- and Mo-MCF viruses differ in only one (X45) of the seven large oligonucleotides (X7, X10, X16, X21, X22/M59, X31, and X45) residing in or very near the env gene sequences, which is consistent with <1% mismatching. Comparisons of the published nucleotide sequences of MCF viruses (17-22) reveal only three base differences in the sequences corresponding to the seven oligonucleotides. Two of the differences reside in adjacent bases in the same MCF genome (22) and result in the generation of X45. These sequence data seem to indicate that the various MCF env sequences are similar enough to be all derived from the same endogenous proviral sequences, assuming that minor base differences in the viral sequences are the result of random mutation. However, the recovery of oligonucleotide X45 in multiple Mo-MCF virus isolates indicates that this oligonucleotide is not the result of random mutation but is present in the endogenous sequence giving rise to the Mo-MCF viruses. Similarly, the absence of this oligonucleotide from one Fr-MCF virus isolate indicates that it is not a component of the endogenous sequences giving rise to these viruses. Additional oligonucleotide differences that distinguish the endogenous sequences of Fr- and Mo-MCF viruses are found in the 3' region of the pol gene (Fig. 3).

It is also interesting that the prevalent patterns of recombination seen with each of the three endogenous sequences are distinctly different (Fig. 3).

Our observation that the Mo-MCF viruses are oncogenic, whereas our Fr-MCF viruses are not, suggests that recombination with a particular env sequence may influence the oncogenicity of a recombinant virus. Preliminary analyses of MCF viruses derived from another strain of Fr-MuLV showed that these MCF viruses are antigenically similar to the Mo-MCF viruses (unpublished observations). It should be of interest to determine if these MCF viruses originate from the same endogenous sequences as the Mo-MCF viruses and to examine their oncogenicity.