NOTES

Comparative Analysis of the Genomes of Feline Leukemia Viruses

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The genomes of several strains of feline leukemia virus (FeLV) were compared by two-dimensional polyacrylamide gel electrophoresis of the large RNase T₁-resistant oligonucleotides of the 70S RNA. Differences between each strain of FeLV tested were detected by this method. We estimate that the degree of sequence identity between the viruses is: FeLV A (Glasgow-1) to FeLV B (Snyder-Theilen), 52%; FeLV A (Glasgow-1) to FeLV C (Sarima), 66%; FeLV B (Snyder-Theilen) to FeLV C (Sarima), 37%. The fingerprints of two independent isolates of FeLV strains of subgroup A (Glasgow-1 and Rickard) were detectably different. We conclude that the RNase T₁ oligonucleotide fingerprint pattern provides a useful tool for identification of FeLV strains.

Feline leukemia viruses (FeLV's) are a horizontally transmitted group of type C retroviruses that cause lymphoma, lymphoblastic leukemia, fibrosarcoma, and aplastic anemia in cats (1, 2). This class of viruses is horizontally transmitted, as demonstrated by epidemiological studies of multiple-cat households (3) and by experimental infections under laboratory conditions (4, 5). The FeLV's have been classified into three distinct subgroups, A (FeLV A), B (FeLV B), and C (FeLV C), on the basis of type-specific neutralization and interference tests (14, 15). Tests of homology by nucleic acid hybridization show that these viruses are closely related. At least 85% of the complementary DNA prepared from any one of the subgroups will anneal to any of the other subgroups. Moreover, the melting temperature of the hybrids formed between the complementary DNA of one subgroup and the RNA of another is within 1°C of that of the homologous hybrids (10).

Although the FeLV's appear to be very closely related by hybridization techniques, they differ markedly in their ability to infect heterologous cell lines. Subgroup A viruses replicate primarily in feline cells, whereas viruses of subgroups B and C replicate additionally in human and mink cells (8, 16). These viruses also differ in their natural patterns of occurrence. All field isolates of FeLV contain FeLV A either alone or in a mixture of subgroups (8, 15). FeLV C is rarely isolated in nature and has never been detected

in the absence of other FeLV subgroups (7). In diseased cats under natural conditions, no observed correlation exists between the FeLV subgroup isolated and the type of disease that is produced (7).

The differences in the biology of the FeLV's motivated us to investigate the structure of the viral genomes using a method capable of detecting differences between closely related viruses. For this study, we used a high-resolution two-dimensional RNA fingerprinting technique which permits direct comparison of approximately 15% of the viral genome (11, 12). Genomic 70S RNA was prepared from virus strains propagated in fibroblasts. The fingerprints of three prototype strains, FeLV A (Glasgow-1), FeLV B (Snyder-Theilen), and FeLV C (Sarima), are shown in Fig. 1.

About 60 large RNase T₁-resistant oligonucleotides were identified in the fingerprint of each virus. The unique oligonucleotides are identified as those species that migrate slowly in the second dimension and that appear to be present in unimolar amounts as judged by the amount of radioactivity in the gel corresponding to the individual oligonucleotides. Our previous studies indicate that the labeling methods used here result in uniform labeling of the oligonucleotides (11, 12). The complexity of the fingerprint patterns for these three viruses is that anticipated if each isolate were a pure single species and not a mixture of two or more virus strains.

FIG. 1. Autoradiograms of the two-dimensional RNA fingerprints of FeLV subgroups. Viruses and cells were provided by O. Jarrett. FeLV A (Glasgow-1) was isolated from a cat with spontaneous alimentary lymphosarcoma (9). FeLV B (Snyder-Theilen) was purified from a mixture of subgroups A and B present as the helper viruses in the Snyder-Theilen strain of FeSV (14). FeLV C (Sarma) was purified from a mixture of subgroups A and C isolated from FL-237 cells (14). Virus was harvested from supernatant culture fluids collected from confluent roller bottles once or twice daily and concentrated by ultracentrifugation. Virus pellets were then suspended and pelleted through 30% sucrose in TNE (50 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA), followed by

inspection of Fig. 1 reveals some similarities and some differences in the fingerprint patterns of each strain. To obtain qualitative estimates of the similarity in the fingerprints, equal amounts of ^{32}P -labeled oligonucleotides derived from each virus were mixed in pairs and then analyzed by two-dimensional gel electrophoresis. To determine the number of unique oligonucleotides that had the same electrophoretic mobility, the fingerprint pattern of the mixture was then compared with independent fingerprints of each virus.

A schematic representation of one such experiment is pictured in Fig. 2A for FeLV A (Glasgow-1) and FeLV B (Snyder-Theilen). The unique oligonucleotides that have the same mobility in the two virus strains are indicated as solid circles, those that are unique to FeLV A (Glasgow-1) are indicated by open circles, and those that are unique to FeLV B (Snyder-Theilen) are indicated by shaded circles. Of the 61 unique oligonucleotides of FeLV A (Glasgow-1), 32 (52%) comigrate with those of FeLV B (Snyder-Theilen), and 32 of the 53 unique oligonucleotides of FeLV B (Snyder-Theilen) comigrate with those of FeLV A (Glasgow-1).

The extent of comigration permits a rough estimation of the sequence identity of the two viral strains (Table 1). The estimate assumes that the sequence identity of the oligonucleotides is representative of the sequence identity of the entire genome. Calculations based on comigration may slightly overestimate the degree of sequence identity, since oligonucleotides which comigrate may have the same base com-

digestion with proteinase K (E.M. Biochemicals). 70S viral RNA-containing fractions, pooled from a linear 15 to 30% sucrose gradient in TNE plus 0.2% sodium dodecyl sulfate, were passed over an oligodeoxythymidylic acid-cellulose column (Collaborative Research, T-3). The polyadenylic acid-containing 70S RNA was eluted with 10 mM Tris-hydrochloride (pH 7.5), precipitated twice with ethanol, and suspended in 20 mM Tris (pH 8.0)-2 mM EDTA. *In vitro* ^{32}P labeling of viral RNA was performed according to the method of Pedersen and Haseltine (11, 12). A 0.2- to 0.4- μg sample of viral RNA was digested with RNase T₁ (Calbiochem) and bacterial alkaline phosphatase (RNase free; Worthington Biochemical Co.). The digested RNA was subsequently labeled with [γ - ^{32}P]ATP and polynucleotide kinase (P.L. Biochemicals). The γ - ^{32}P -labeled oligonucleotides were electrophoresed in a first-dimension 10% polyacrylamide gel containing 6 M urea and 25 mM citric acid (pH 3.5), followed by electrophoresis in a second-dimension 22.5% polyacrylamide gel containing 50 mM Tris, 50 mM boric acid, and 1 mM EDTA (pH 8.3). The gel was then autoradiographed. (A) FeLV A (Glasgow-1); (B) FeLV B (Snyder-Theilen); (C) FeLV C (Sarma).

FIG. 2. Comparison of the genomes of FeLV. Equal amounts of labeled RNase T₁-resistant oligonucleotides from two different strains were mixed together immediately before electrophoresis in the first dimension. Two-dimensional gel electrophoresis was performed as previously described. (A) Schematic representation of the mixture of FeLV A (Glasgow-1) and FeLV B (Snyder-Theilen). RNase T₁-resistant oligonucleotides: (open circles) found only in FeLV A; (shaded circles), found only in FeLV B; (black circles), common to both FeLV A and FeLV B. (B) Schematic representation of the mixture of FeLV A (Glasgow-1) and FeLV C (Sarma). RNase T₁-resistant oligonucleotides: (open circles) found only in FeLV C; (shaded circles), found only in FeLV A; (black circles), common to both FeLV A and FeLV C. (C) Schematic representation of the mixture of FeLV B (Snyder-Theilen) and FeLV C (Sarma). RNase T₁-resistant oligonucleotides: (open circles) found only in FeLV C; (shaded circles) found only in FeLV B; (black circles) common to both FeLV B and FeLV C.

TABLE 1. *Calculated sequence identities of the FeLV's*

Viral genome	No. (%) showing sequence identity with viral genome ^a			
	FeLV A (Glasgow-1)	FeLV B	FeLV C	FeLV A (Rickard)
FeLV A (Glasgow-1)	59/59 (100)	32/61 (52)	37/58 (64)	46/60 (76)
FeLV B (Snyder-Theilen)	32/53 (60)	55/55 (100)	21/56 (37)	ND
FeLV C (Sarma)	37/56 (66)	21/56 (37)	56/56 (100)	ND
FeLV A (Rickard)	46/59 (78)	ND	ND	60/60 (100)

^a Number of RNase T₁-resistant oligonucleotides found in common between the two viral genomes/total number of unique oligonucleotides. The number of unique oligonucleotides scored for a particular viral strain varied slightly from experiment to experiment as a result of the degree of resolution of each fingerprint. The numbers in parentheses are the percentages of sequence identity. ND, Not done.

position but not necessarily the same sequence. On the other hand, this calculation may be an underestimate, since the mobility of an oligonucleotide can be altered by a change in a single nucleotide.

Similar analysis was done on mixtures of ³²P-labeled oligonucleotides of FeLV A (Glasgow-1) and FeLV C (Sarma) (Fig. 2B) and of FeLV B (Snyder-Theilen) and FeLV C (Sarma) (Fig. 2C). The number of comigrating oligonucleotides was determined as described above and is listed in Table 1 for each experiment. We estimate that 64 to 66% of the sequences of FeLV A (Glasgow-1) and FeLV C (Sarma) are identical. The genomes of FeLV B (Snyder-Theilen) and FeLV C (Sarma) are more distantly related. Only about one-third (37%) of the unique oligonucleotides have the same electrophoretic mobility.

To determine whether the fingerprint of an FeLV strain was affected by the species of fibroblasts on which the virus was grown, the fingerprint of FeLV B (Snyder-Theilen) grown on feline fibroblasts was compared with that of the same virus grown on canine fibroblasts. No differences in the fingerprints of the two preparations were detected in this experiment (not shown).

The preceding experiments demonstrate that viruses of the different subgroups can be distinguished from one another by analysis of the unique large RNase T₁-resistant oligonucleotides. To determine whether viruses of the same subgroup could also be distinguished from each other by this method, the fingerprint of the viral RNA of FeLV A (Glasgow-1) was compared with that of another subgroup A virus, FeLV A (Rickard) (Fig. 3). We estimate that the degree of sequence identity between these two strains is

FIG. 3. Comparison of the two subgroup A virus genomes. FeLV A (Rickard) was isolated from F422 cells, a spontaneous lymphoid cell line provided by M. Essex (13). (A) FeLV A (Glasgow-1); (B) FeLV A (Rickard); (C) Schematic diagram. RNase T₁-resistant oligonucleotides; (open circles) found only in FeLV A (Rickard); (shaded circles) found only in FeLV A (Glasgow-1); (black circles) common to both.

76 to 78% (Table 1). It is possible that the background spots on the autoradiogram of the fingerprint of FeLV A (Rickard) represent sequences from a second viral or subviral component.

The extent of sequence identity detected among the FeLV strains was anticipated from previous studies of nucleic acid homology. These studies indicated an overall homology of about 85% among all FeLV strains tested. This homology is consistent with the degree of sequence identity repeated here, with the possible exception of the relatively low degree of identity, 37%, found between FeLV B (Snyder-Theilen) and FeLV C (Sarma).

The results presented here demonstrate that a fingerprint of the 70S genomic RNA provides a useful method for identifying an FeLV strain. No two FeLV strains tested had the same fingerprint, including two independent subgroup A isolates. The fingerprints presented here should provide a standard for future FeLV identification. The subgroup type of each of the isolates used here was confirmed in blind tests of subgroup specificity by Oswald Jarrett.

As an example of the utility of this method for virological studies of FeLV, we have used the RNA fingerprinting technique to correctly identify the FeLV helper component of a mixture of FeLV and defective feline sarcoma virus genomes as belonging to subgroup B. The helper virus had been previously thought to belong to subgroup C. We have also fingerprinted a virus described as subgroup B and detected a second subgroup A viral component in the analysis. This virus was subsequently typed as two viruses with both subgroup A and subgroup B specificities. (A preliminary report of this work has been published [6]).

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