Characterization of Structural and Immunological Properties of Specific Domains of Friend Ecotropic and Dual-Tropic Murine Leukemia Virus gp70s

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A detailed comparison of the gp70 proteins of cloned ecotropic Friend murine leukemia virus (FLV) and dual-tropic Friend mink focus-forming virus (FrMCF) was performed by analyzing the structural and immunological properties of amino- and carboxy-terminal domains of these molecules generated upon controlled trypsinization. The two gp70s gave characteristic fragmentation patterns; the amino-terminal fragments of FrMCF gp70 were smaller than the corresponding fragments of FLV and contained a trypsin site which resulted in a 19,000-dalton amino-terminal fragment not observed for FLV, whereas both molecules yielded an identically sized carboxy-terminal fragment. All amino-terminal fragments of both gp70 molecules contained an endo H-sensitive oligosaccharide chain; for FrMCF, a second endo H-sensitive carbohydrate was present as well at a carboxy-terminal site for approximately 50% of the molecules. Several aspects of the disulfide interactions of the two gp70s were conserved; in both cases the carboxy-terminal fragments were disulfide bonded to p15(E), there were no disulfide bonds between amino- and carboxy-terminal fragments, and the amino-terminal fragments exhibited a significant increase in mobility upon analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions. Analysis of the immunoreactivity of the different domains of the proteins by immunoprecipitation of the fragments with antisera prepared against xenotropic murine leukemia virus and feline leukemia virus gp70s indicated major differences in antigenicity for the amino-terminal domains of FLV and FrMCF gp70, whereas the carboxy-terminal domains were immunologically conserved. Similar analyses with antibodies specific for p15(E) and Pr15(E) demonstrate that these components are conserved as well. These data provide direct evidence that p15(E) and the C-terminal gp70 domain of FrMCF gp70 are related to the corresponding regions of the ecotropic FLV parent and indicate that the acquisition of MCF-specific properties is due to the replacement of the ecotropic amino-terminal gp70 domain with sequences related to those of xenotropic gp70s.

The original Friend virus isolate consists of a complex between a replication-competent ecotropic virus, Friend murine leukemia virus (FLV), and a replication-defective virus, spleen focus-forming virus (28). Whereas the spleen focus-forming virus component appears to be responsible for some of the biological properties of the complex, it has been shown with both biologically and molecularly cloned viruses that infection with FLV by itself results in rapid transformation of hematopoietic cells of the erythroid lineage (19, 31), and evidence has been presented which suggests that FLV leukemogenesis proceeds via a recombinant mink cell focus-forming (MCF) virus intermediate. FLV-induced leukemias contain greatly increased levels of xenotropic murine leukemia virus (MuLV)-related *env* sequences (31), and MCF viruses have frequently been isolated from such leukemic spleens (10, 31). These MCF isolates are themselves leukemogenic in newborn mice (10) and in adult mice when inoculated as pseudotypes with nonpathogenic viruses (24). In addition, certain strains of mice, such as DBA/2, which are resistant to infection by MCF viruses are resistant to leukemogenesis by FLV (1, 24). Thus, it appears that, as has been shown for the AKR system, recombinant dual-tropic viruses may be the proximal leukemogenic agents in FLV-induced disease.

We are interested in determining the structural features of the MCF gp70 molecules which correlate with their broadened host range and enhanced pathogenicities. Towards this aim, we have recently described conditions for generating and characterizing fragments of native gp70s which correspond to specific amino- and carboxy-terminal domains of the molecules (21, 22). Applying this technique to the study of ecotropic Akv gp70 and its leukemogenic recombinant, MCF-247, we have found that these two gp70s differ extensively in their amino-terminal regions but possess conserved carboxy-terminal domains (21). In the present study, we use a similar approach to compare the structural and immunological properties of FLV and Friend MCF (FrMCF) gp70 domains. Our results indicate that, in the Friend system as well, major structural and immunological differences exist in the amino-terminal domains of the ecotropic and dual-tropic viral gp70s, whereas the carboxy-terminal domains appear to be highly related. We find that the amino-terminal domain of FrMCF is immunologically related to xenotropic gp70s of both the BV-2 and NZB classes of MuLV and antigenically distinct from the corresponding region of ecotropic FLV. These results, consistent with the previous localization of a xenotropic-related antigenic determinant to a 23,000-dalton amino-terminal fragment of FrMCF gp70 (33), suggest that the amino-terminal domain of gp70 is functionally important in determining the receptor specificity of these viruses.

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MATERIALS AND METHODS

Viruses. NIH 3T3 cell cultures infected with molecularly cloned ecotropic FLV, clone 57, and biologically cloned FrMCF-1 virus were obtained from Allen Oliff. Viruses were generally labeled by culturing cells overnight in cysteine- or methionine-free minimum essential medium supplemented with 100 to 150 μ Ci of 35 S-labeled cysteine or methionine per ml; labeling with [3 H]mannose was performed by culturing cells overnight in minimum essential medium containing 500 μ Ci of [3 H]mannose per ml. Virions were purified by banding directly on 15 to 60% sucrose gradients.

Conditions for fragmentation and immunoprecipitation of gp70. Virions were solubilized by addition of equal volumes of $2\times$ RIP buffer (RIP buffer = 0.01 M Tris [pH 7.4], 0.5 M NaCl, 0.5% Nonidet P-40) and treated with appropriate concentrations of trypsin for 15 min at 37°C, followed by inactivation of the trypsin by addition of 1,000 U of Trasylol (FBA Pharmaceuticals) per ml. For experiments involving intact virions, freshly purified, unfrozen virus preparations were trypsinized, treated with Trasylol, and then solubilized with RIP buffer. Viral lysates were precleared by incubation with *Staphylococcus aureus* (Pansorbin; Calbiochem), and nonspecifically bound components were removed by pelleting. Immunoprecipitations were then performed by incubation for 1 h at 37°C with antisera diluted 1:100, and immune complexes were collected with Pansorbin and washed three times with RIP buffer. Samples were solubilized by boiling for 1 min in buffer containing 1% sodium dodecyl sulfate (SDS) and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) on 10 or 12% polyacrylamide gels, as described by Laemmli (13). For samples analyzed under reducing conditions, 1% dithiothreitol was included in the SDS sample buffer. Gels were treated for fluorography as described by Laskey and Mills (14) and exposed to X-ray film at -70°C.

Reagents and antisera. Trypsin (tolylsulfonil phenylalanyl

chloromethyl ketone treated; 12,100 BAEE units per mg of protein) was obtained from Miles Laboratories. Radioisotopes were purchased from New England Nuclear Corp. The following antisera were obtained from the Biological Carcinogenesis Branch of the National Cancer Institute: goat α Rauscher gp69/71, lot no. 5S-617 and 78S-225 (the latter serum contained a proteolytic activity which was inactivated by heating at 50°C for 1 h); goat α BV2 gp70, lot no. 76S-431; goat α -feline leukemia virus (FeLV) gp70, lot no. 7341. Rabbit α XenCSA serum, prepared by immunization with rabbit SIRC cells infected with NZB-IU-1 MuLV (17), was obtained from H. C. Morse, Jr. This serum recognized both gp70 and p15(E). Monoclonal α p15(E) antibodies 9-E8 (16) and 42/114 (20) have been previously described. Rabbit α R serum, prepared against a synthetic pentadecapeptide corresponding to the C terminus of gPr80^{env} and Pr15(E) of Moloney MuLV (7, 27), was obtained from R. Lerner.

RESULTS

Comparison of FLV and FrMCF gp70 structural domains.

The fragmentation patterns resulting from treatment of solubilized gp70s of ecotropic and dual-tropic Friend MuLVs with various concentrations of trypsin are illustrated in Fig. 1. For [35 S]cysteine-labeled FLV gp70, four major fragments were obtained after trypsinization, with SDS-PAGE mobilities indicating apparent molecular weights of 51,000, 39,000, 36,000, and 34,000 (Fig. 1A); a fainter band of 53K is occasionally seen. The 51K band, and to a lesser extent the 36K band, was quite sensitive to further digestion by trypsin, whereas the 39K and 34K components were relatively stable. The 39K band was considerably broader than the other components, suggesting more extensive glycosylation. Analysis of the fragmentation pattern of FLV gp70 labeled with [35 S]methionine indicated that the 39K and 53K fragments do not contain any methionine, whereas the other three components do. Since it has been shown that FLV

FIG. 1. Analysis of trypsin-generated fragments of ecotropic and dual-tropic Friend gp70s by SDS-PAGE. Solubilized [35 S]cysteine and methionine-labeled FLV (A), or [35 S]cysteine-labeled FrMCF (B), was immunoprecipitated after treatment with the following concentrations of trypsin: 1 (control), no trypsin; 2, 2 μ g/ml; 3, 10 μ g/ml; 4, 50 μ g/ml. FLV samples were immunoprecipitated with α Rauscher gp70 serum. The lane marked "v" contained a [35 S]cysteine-labeled FLV marker. FrMCF samples were immunoprecipitated as indicated with either α gp70 serum or monoclonal α p15(E) antibody 9-E8.

gp70 contains a single methionine residue at position 47 from the amino terminus of the mature molecule (3, 12), this indicates that the 51K, 36K, and 34K fragments must be derived from the amino-terminal end of the molecule and that the other two bands are carboxy-terminal fragments. In confirmation of these assignments, when similar samples were analyzed under nonreducing conditions, the 39K fragment and the p15(E) band were not detected, and a new band corresponding in size to the disulfide-linked 39K-p15(E) complex was observed (Fig. 2A). This is consistent with our previous demonstration for Akv and MCF-247 MuLVs (21, 22), and with that by others for Rauscher virus (18), that p15(E) is disulfide linked to a site in the carboxy-terminal domain of gp70. Figure 2A also demonstrates that the mobilities of the amino-terminal fragments increase significantly under nonreducing conditions (see also Fig. 5), suggesting that they contain one or more disulfide bonds which strongly affect the conformation of the denatured fragments. We have reported a similar feature for the amino-terminal fragments of Akv and MCF-247 gp70s (21, 22).

The trypsin-generated fragmentation pattern of [35 S]cysteine-labeled FrMCF gp70 is illustrated in Fig. 1B. For this molecule similar patterns were obtained with cysteine- and methionine-labeled viruses, indicating that methionine residues were present in both amino and carboxy domains. Two relatively broad bands of 49K and 39K are formed which can be coprecipitated with α p15(E) sera, suggesting by analogy with the results obtained with ecotropic gp70s that these represent the carboxy-terminal domain of the molecule. The larger of these components is cleaved at higher trypsin concentrations, whereas the 39K band is stable, suggesting that the 49K fragment is a precursor to the 39K component. Additional fragments of 42K, 32K, 29K, and 19K are formed which are not associated with p15(E) and which therefore appear to be amino-terminal fragments. Analysis of the concentrations of these fragments formed with increasing

amounts of trypsin suggests that the larger forms are precursor to the smaller forms, with the 19K fragment being the final product of trypsinization.

The carbohydrate content of the various gp70 fragments was analyzed by examining the effect of treatment with endo H on the mobilities of the fragments and by examining the pattern obtained upon trypsinization of [3 H]mannose-labeled FrMCF. We have previously found that a glycosylation site in the amino-terminal domain of Akv gp70 possesses the interesting feature that it occasionally retains an endo H-sensitive oligosaccharide (22). A similar site is present in the corresponding domain of MCF-247 gp70, and for this virus all of the gp70 molecules possess such as endo H-sensitive carbohydrate chain (21). For FLV gp70 we observed that the amino-terminal 51K, 36K, and 34K fragments completely underwent a size shift upon digestion with endo H, consistent with the removal of a single high-mannose oligosaccharide chain (Fig. 2A). Thus, for FLV all of the gp70 molecules retained an endo H-sensitive amino-terminal oligosaccharide. A similar result was obtained for the FrMCF gp70 amino-terminal fragments (Fig. 2B). All of the FrMCF gp70 fragments detected with [35 S]cysteine were also labeled with mannose, whereas, as expected, p15(E) did not incorporate any of the mannose label. After endo H digestion, the mannose-labeled amino-terminal bands were greatly decreased in intensity, consistent with the removal of most of the mannose residues, whereas the carboxy-terminal bands remained heavily labeled. The deglycosylated amino-terminal fragments did, however, retain a low level of [3 H]mannose label, indicating that these fragments contain an additional oligosaccharide chain, relatively poor in mannose, which is resistant to endo H.

Also apparent from Fig. 2B is that a fraction of the carboxy-terminal fragments of 49K and 39K also underwent a mobility shift after endo H treatment, indicating that for FrMCF gp70 the carboxy-terminal 39K domain also contains

FIG. 2. Analysis of endo H sensitivity of Friend virus gp70 fragments. (A) Solubilized [35 S]cysteine-labeled FLV gp70 was digested with 2 μ g of trypsin per ml, immunoprecipitated with α Rauscher gp70 serum, and redissolved in buffer containing 1% SDS. Lanes marked "+" contained samples subsequently digested with 2 μ g of endo H per ml; lanes marked "-" contained undigested control samples. The resulting products were analyzed under both reducing and nonreducing conditions. (B) Solubilized FrMCF gp70, labeled with either [35 S]cysteine or [3 H]mannose, was digested with 2.5 μ g trypsin (lanes 1 and 2) or 25 μ g trypsin (lanes 3 and 4) per ml, immunoprecipitated with α gp70 serum, and redissolved in buffer containing 1% SDS. Lanes 2 and 4 contained samples subsequently treated with 2 μ g of endo H per ml; samples in lanes 1 and 3 were directly analyzed. All samples were reduced before analysis by SDS-PAGE.

FIG. 3. Analysis of gp70 fragmentation patterns of intact virions. Freshly purified, intact FLV and FrMCF, labeled with [35 S]cysteine, were digested with trypsin, the trypsin was neutralized with Trasylol, and the virions were then solubilized and immunoprecipitated with α Rauscher gp70 serum. The concentrations of trypsin used were: 1, 1 μ g/ml; 2, 10 μ g/ml; 3, 100 μ g/ml; 4, 1,000 μ g/ml; 5, 2 μ g/ml; 6, 10 μ g/ml; 7, 50 μ g/ml; 8, 250 μ g/ml; 9, 1,250 μ g/ml. All samples were analyzed by SDS-PAGE under reducing conditions.

a site which partially retains a high-mannose oligosaccharide chain. From the intensities of the modified and residual bands one can estimate that approximately 50% of the gp70 molecules contain this carboxy-terminal endo H site. This result distinguishes FrMCF from all ecotropic gp70s analyzed to date, as well as from gp70 of the dual-tropic MCF-247 MuLV.

Comparison of fragments produced by trypsinization of lysed and intact virions. The results described above indicate that solubilized gp70s of both FLV and FrMCF contain a limited number of trypsin-sensitive sites which are located at roughly homologous positions for the two proteins. To obtain information on the native conformations of the gp70 molecules in the viral membranes, we examined which of these trypsin sites were exposed in intact virions. In these experiments freshly purified virions were incubated with various concentrations of trypsin, the trypsin was neutral-

ized with Trasylol, the virions were solubilized with RIP buffer, and gp70 fragments were immunoprecipitated. gp70 was considerably more resistant to proteolysis in intact virions than after solubilization (Fig. 3); the intact virions required about 25-fold-higher trypsin concentrations to achieve a similar extent of gp70 cleavage. The resulting patterns differed primarily in that the amino-terminal fragments of 51K for FLV and 42K for FrMCF, which are major initial products for solubilized gp70, are not produced at all for intact virions. Similarly, the carboxy-terminal 39K fragments, which are a major initial product in lysed virions, are formed only at the highest trypsin concentrations for intact virus. The preferred pathway for cleavage of gp70 in intact virions involves trypsinization at amino-terminal sites, resulting in the generation of the small amino-terminal fragments and large carboxy-terminal products. These alternate cleavage pathways are diagrammed in Fig. 4.

Characterization of immunoreactivities of FLV and FrMCF gp70 domains. To obtain information on the antigenic relatedness of the corresponding domains of FLV and FrMCF gp70s, we examined the immunoreactivity of the fragments obtained from these two proteins with antisera prepared against gp70s of xenotropic MuLVs and FeLV. All of the FrMCF domains reacted readily with a high-titered antiserum prepared against gp70 of the xenotropic BV-2 virus (Fig. 5), whereas this serum preferentially recognized the carboxy-terminal fragment of FLV gp70 (lane 2). A second serum specific for xenotropic NZB MuLV gp70, α XenCSA serum (17), specifically recognized the MCF amino-terminal domains and reacted very weakly if at all with the carboxy-terminal fragments of FrMCF and FLV gp70 (lane 3). The α FeLV gp70 serum, on the other hand, reacted quite well with carboxy-terminal FLV gp70 fragments and only weakly with the amino-terminal bands; this serum recognized predominantly the carboxy-terminal fragments of FrMCF (lane 4). These results indicate that the major antigenic differences between the FLV and FrMCF gp70s reside in the amino-terminal domains, whereas the carboxy-terminal domains of these two proteins share multiple conserved antigenic determinants.

The p15(E) proteins of FLV and FrMCF also possess identical mobilities and immunoreactivities. FrMCF p15(E) is recognized by two monoclonal antibodies: 42/114, which reacts with a widely conserved determinant localized in the

FIG. 4. Diagram indicating orientations and structural features of fragments formed upon trypsinization of gp70 from intact and solubilized FLV and FrMCF.

amino-terminal half of p15(E) (20); and 9-E8, which reacts with an ecotropic-specific p15(E) determinant (16) (Fig. 6, lanes 2 and 3). In addition, the p15(E) band of FrMCF is recognized by α R serum, prepared against the C-terminal 15 amino acids ("R" peptide) of the Moloney MuLV *env* gene product (27) (Fig. 6, lane 4). This is a highly type-specific serum which has previously been reported to react only with Moloney virus and not with endogenous MuLVs (7). The sequence of the R peptide of FLV is highly related to that of Moloney MuLV, differing only in the substitution of a tyrosine for a phenylalanine and a leucine for an isoleucine (12, 27), thereby accounting for this cross-reactivity. These results are consistent with the proposed recombinant nature of FrMCF and suggest that the genetic sequences coding for the carboxy-terminal gp70 domain and p15(E) of FrMCF are derived from FLV.

DISCUSSION

The structural characteristics of FLV and FrMCF gp70s and the origin of the fragments generated upon trypsinization of both intact and solubilized virions are summarized diagrammatically in Fig. 4. The position of the single methionine residue of FLV gp70 determined by DNA and protein sequencing to be a residue 47 is indicated by the circled M. Both viruses contain an endo H-sensitive oligosaccharide chain in their amino-terminal fragments. Comparison of the sequences of several ecotropic gp70s (12, 15, 26) with those of MCF-247 (9) and the Moloney MCF81 isolate (2) indicates that the only conserved glycosylation site in the amino-terminal domains of these viruses is the one at residue 12 of the ecotropic gp70s. This site is conserved as well for the NFS-Th-1 xenotropic gp70 (23) and is also present in a molecularly cloned isolate of FrMCF gp70 (A. Oliff and R. Friedrich, personal communication). Thus, it is most likely that this is the site which contains the endo H-sensitive carbohydrates; this site is indicated as E-CHO on the two

gp70 maps. There are no trypsin-sensitive residues between this site and the amino terminus of the molecule, and thus this oligosaccharide serves as a marker for that end of the molecule. Our data also indicate a carboxy-terminal site in FrMCF gp70 which retains an endo H-sensitive oligosaccharide chain approximately 50% of the time. This site is also indicated in Fig. 4; the exact location of this site within the 39K fragment is not known.

Sequencing data for both ecotropic and MCF gp70s indicate that these molecules are composed of cysteine-rich amino- and carboxy-terminal domains, joined by a cysteine-free, proline-rich region which is hypervariable for several ecotropic gp70s (2, 12, 15, 26). Our data indicate that there are no disulfide interactions between the amino- and carboxy-terminal domains of either class of gp70 and allows the preliminary localization of several disulfide bonds which are conserved for the two viruses. The disulfide linkage site to p15(E) for both viruses is located in the 39K carboxy-terminal domain, and both gp70s contain one or more disulfide bonds in their amino-terminal domains which result in a significant increase in the electrophoretic mobility of fragments derived from this region upon analysis under nonreducing conditions. For FrMCF gp70 we have observed that the 19K amino-terminal fragment does not exhibit this effect (Fig. 5), and thus we can localize the bonds involved to the region between 19K and 29K from the amino terminus. This is so indicated in the FrMCF gp70 map as a single disulfide bond. The conservation of this structural feature for both classes of gp70 suggests that it may be required to maintain a functionally important conformation.

The gp70 fragments obtained upon limited trypsinization can be accounted for by postulating three primary cleavage sites for FLV and four for FrMCF gp70. The apparently homologous sites for the two viruses are numbered 1, 2, and 3. In comparing the cleavage patterns of gp70 from intact and solubilized virions, the most striking difference is that cleav-

FIG. 5. Analysis of immunoreactivities of FLV and FrMCF gp70 fragments. [35 S]cysteine-labeled virions were solubilized and digested with 2 μ g of trypsin per ml, and the resulting fragments were immunoprecipitated with the following antisera: 1, α Rauscher gp70 serum; 2, α BV-2 gp70 serum; 3, α XenCSA serum; 4, α FeLV gp70 serum; 5, normal goat serum. The immunoprecipitates were analyzed under both reducing and nonreducing conditions.

FIG. 6. Analysis of immunoreactivities of FrMCF p15(E) and Pr15(E). [35 S]methionine-labeled FrMCF proteins were immunoprecipitated with α Rauscher gp70 serum (lane 1), monoclonal α p15(E) antibody 42/114 (lane 2), monoclonal α p15(E) antibody 9-E8 (lane 3), α R serum (lane 4), or normal goat serum (lane 5) and analyzed by SDS-PAGE after reduction.

age in the carboxy-terminal domain at site 1 is markedly inhibited in intact virions, so that the large amino-terminal fragments, which are major preliminary products for solubilized gp70s, are not formed at all and the smaller 39K carboxy-terminal fragments are only formed at very high trypsin concentrations. These results indicate that this gp70 site is not accessible in the intact oligomeric *env* protein complex, suggesting that this region may be closely associated with the viral membrane, p15(E) molecules, or regions involved in gp70 oligomerization. Possibly relevant to this feature of gp70 is our recent demonstration of occasional disulfide bonding between the carboxy-terminal domains of neighboring gp70 molecules for Akv and MCF-247 MuLVs (21).

The immunological studies illustrated in Fig. 5 demonstrate that the amino-terminal domain of FrMCF gp70 is readily recognized by antisera to gp70s of both BV-2 and NZB xenotropic MuLVs, whereas the corresponding domain of ecotropic FLV gp70 is antigenically unrelated. The BV-2 and NZB viruses are prototypes for two serologically distinguishable classes of xenotropic MuLVs: the class II viruses inducible by halogenated pyrimidines and protein synthesis inhibitors from cells of a large number of mouse strains, and the class III viruses which are spontaneously expressed at high levels by cells from NZB mice (29, 30). The latter gp70 is related to the gp70 found in the sera of mice of a large number of strains (5). These two classes of xenotropic gp70s can be distinguished by both peptide mapping (4) and type-specific radioimmunoassays (8). The reactivity of both antisera with the amino-terminal FrMCF gp70 fragments indicates that this region of the molecule contains antigenic determinants in common with both classes of xenotropic gp70s. The reactivity of the α Rauscher gp70 serum with the amino-terminal domains of both ecotropic and MCF viruses probably indicates that this serum was actually prepared against a mixture of ecotropic and dual-tropic gp70s. Supporting this explanation is our observation that the anti-xenotropic gp70 sera do not precipitate the amino-terminal domain of ecotropic Rauscher gp70 (data not shown) and the recent demonstration that the gp69 compo-

nent of the gp69/71 protein complex produced by Rauscher-infected JLSV-9 cells represents a dual-tropic gp70 molecule (25). The preferential reactivity of the α FLV gp70 serum with the C-terminal fragments of FLV and FrMCF gp70 provides further demonstration of the conserved nature of this region and indicates that this domain carries the major interspecies-specific antigenic determinants of gp70 molecules.

Whereas the data presented in this paper indicate that the carboxy-terminal domains of FLV and FrMCF-1 gp70 are similar in size and immunologically cross-reactive, these regions are not identical in the two viruses, as evidenced by the fact that the C-terminal fragment of FrMCF gp70, but not of FLV gp70, contains a glycosylation site which occasionally retains an endo H-sensitive oligosaccharide (Fig. 2). This difference in glycosylation may reflect an altered conformation for this region in the MCF protein, perhaps influenced by association with the xenotropic-like amino-terminal domain, or may be due to differences in primary structure of the C-terminal domains of the two gp70s. Sequence information will be useful for distinguishing between these possible explanations.

The leukemogenic MCF viruses studied to date can be placed into two classes according to the specificity of the diseases they produce. One class, typified by the Akv and Moloney MuLV recombinants, produces thymic leukemias, whereas the other class, represented by Friend and Rauscher recombinants, results predominantly in leukemias of erythroid origin. The viral regions which determine this disease specificity are not yet known, although considerable evidence suggests that MCF-specific gp70 sequences are required for pathogenicity (6). Comparison of the gp70 fragmentation patterns obtained in this study for FrMCF with those we have previously obtained for the Akv recombinant MCF-247 (20) indicates that the amino-terminal domains of these two dual-tropic gp70s can be distinguished by both the size and the number of fragments generated. This suggests that a different noncancerous parent may have been involved in the generation of these two MCF viruses. More detailed analyses of the structure of the different gp70 domains in these and in other recombinant MuLVs should help clarify the functional importance of these regions of gp70 in determining the pathogenicities and the disease specificities of these viruses.

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LITERATURE CITED

1. Bassin, R. H., S. Ruscetti, I. Ali, D. K. Haapala, and A. Rein. 1982. Normal DBA/2 mouse cells synthesize a glycoprotein which interferes with MCF virus infection. *Virology* **123**:139-151.
2. Bosselman, R. A., F. Van Straaten, C. Van Beveren, I. M. Verma, and M. Vogt. 1982. Analysis of the *env* gene of a molecularly cloned and biologically active Moloney mink cell focus-forming proviral DNA. *J. Virol.* **44**:19-31.
3. Chen, R. 1982. Complete amino acid sequence and glycosylation sites of glycoprotein gp71A of Friend murine leukemia

- virus. *Proc. Natl. Acad. Sci. U.S.A.* **79**:5788–5792.
4. Elder, J. H., J. W. Bautsch, F. C. Jensen, R. A. Lerner, T. M. Chused, H. C. Morse, J. W. Hartley, and W. P. Rowe. 1980. Differential expression of two distinct xenotropic viruses in NZB mice. *Clin. Immunol. Immunopathol.* **15**:493–501.
 5. Elder, J. H., F. C. Jensen, M. L. Bryant, and R. A. Lerner. 1977. Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion associated and differentiation antigens encoded by a multi-gene family. *Nature (London)* **267**:23.
 6. Famulari, N. G. 1983. Murine leukemia viruses with recombinant *env* genes: a discussion of their roles in leukemogenesis. *Curr. Top. Microbiol. Immunol.* **103**:75–101.
 7. Green, N., T. M. Shinnick, O. Witte, A. Ponticelli, J. G. Sutcliffe, and R. A. Lerner. 1981. Sequence-specific antibodies show that maturation of Moloney leukemia virus envelope polypeptide involves removal of a COOH-terminal peptide. *Proc. Natl. Acad. Sci. U.S.A.* **78**:6023–6027.
 8. Hino, S., J. R. Stephenson, and S. A. Aaronson. 1976. Radioimmunoassays for the 70,000-molecular-weight glycoproteins of endogenous mouse type C viruses: viral antigen expression in normal mouse tissues and sera. *J. Virol.* **18**:933–941.
 9. Holland, C. A., J. Wozney, and N. Hopkins. 1983. Nucleotide sequence of the gp70 gene of murine retrovirus MCF 247. *J. Virol.* **47**:413–420.
 10. Ishimoto, A., A. Adachi, K. Sakai, T. Yorifuji, and S. Tsuruta. 1981. Rapid emergence of mink cell focus-forming (MCF) virus in various mice infected with NB-tropic Friend virus. *Virology* **113**:644–655.
 11. Kelley, M., C. A. Holland, M. L. Lung, S. K. Chattopadhyay, D. R. Lowy, and N. H. Hopkins. 1983. Nucleotide sequence of the 3' end of MCF 247 murine leukemia virus. *J. Virol.* **45**:291–298.
 12. Koch, W., G. Hunsmann, and R. Friedrich. 1983. Nucleotide sequence of the envelope gene of Friend murine leukemia virus. *J. Virol.* **45**:1–9.
 13. Laemmli, V. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680–685.
 14. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of ^3H and ^{14}C polyacrylamide gels by fluorography. *Eur. J. Biochem.* **56**:335–341.
 15. Lenz, J., R. Crowther, A. Straceski, and W. Haseltine. Nucleotide sequence of the Akv *env* gene. *J. Virol.* **42**:519–529.
 16. Lostrom, M. E., M. R. Stone, M. Tam, W. N. Burnette, A. Pinter, and R. C. Nowinski. 1979. Monoclonal antibodies against murine leukemia viruses: identification of six antigenic determinants on the p15(E) and gp70 envelope proteins. *Virology* **98**:336–350.
 17. Morse, H. C., T. M. Chused, M. Boehm-Truitt, B. J. Mathieson, S. O. Sharrow, and J. W. Hartley. 1979. XenCSA: cell surface antigens related to the major glycoprotein (gp70) of xenotropic murine leukemia viruses. *J. Immunol.* **122**:443–454.
 18. Niman, H. L., and J. H. Elder. 1982. Structural analysis of Rauscher virus gp70 using monoclonal antibodies: sites of antigenicity and p15(E) linkage. *Virology* **123**:187–205.
 19. Oliff, A. I., G. L. Hager, E. H. Chang, E. M. Scolnick, H. W. Chan, and D. R. Lowy. 1980. Transfection of molecularly cloned Friend murine leukemia virus DNA yields a highly leukemogenic helper-independent type C virus. *J. Virol.* **33**:475–486.
 20. Pinter, A., and W. J. Honnen. 1983. Topography of murine leukemia virus envelope proteins: characterization of transmembrane components. *J. Virol.* **46**:1056–1060.
 21. Pinter, A., and W. J. Honnen. 1983. Comparison of structural domains of gp70 of ecotropic Akv and its dualtropic recombinant MCF-247. *Virology* **129**:40–50.
 22. Pinter, A., W. J. Honnen, J. S. Tung, P. V. O'Donnell, and U. Hammerling. 1982. Structural domains of endogenous murine leukemia virus gp70s containing specific antigenic determinants defined by monoclonal antibodies. *Virology* **116**:499–516.
 23. Repaske, R., R. R. O'Neill, A. S. Khan, and M. A. Martin. 1983. Nucleotide sequence of the *env*-specific segment of NFS-Th-1 xenotropic murine leukemia virus. *J. Virol.* **46**:204–211.
 24. Ruscetti, S., L. Davis, J. Feild, and A. Oliff. 1981. Friend murine leukemia is associated with the formation of mink cell focus-inducing viruses and is blocked in mice expressing endogenous xenotropic viral envelope genes. *J. Exp. Med.* **154**:907–920.
 25. Schultz, A., A. Rein, L. Henderson, and S. Oroszlan. 1983. Biological, chemical, and immunological studies of Rauscher ecotropic and mink cell focus-forming viruses from JLS-V9 cells. *J. Virol.* **45**:995–1003.
 26. Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe. 1981. Nucleotide sequence of Moloney murine leukemia virus. *Nature (London)* **293**:543–548.
 27. Sutcliffe, J. G., T. M. Shinnick, N. Green, F. T. Liu, H. L. Niman, and R. A. Lerner. 1980. Chemical synthesis of a polypeptide predicted from nucleotide sequence allows detection of a new retroviral gene product. *Nature (London)* **287**:801–805.
 28. Steeves, R. A. 1975. Spleen focus-forming virus in Friend and Rauscher leukemia virus preparations. *J. Natl. Cancer Inst.* **54**:289–297.
 29. Stephenson, J. R., S. A. Aaronson, P. Arnstein, R. J. Huebner, and S. R. Tronick. 1974. Demonstration of two immunologically distinct xenotropic type C RNA viruses of mouse cells. *Virology* **61**:56–63.
 30. Stephenson, J. R., R. K. Reynolds, S. R. Tronick, and S. A. Aaronson. 1975. Distribution of three classes of endogenous type-C RNA viruses among inbred strains of mice. *Virology* **67**:404–414.
 31. Troxler, D. H., and E. M. Scolnick. 1978. Rapid leukemia induced by cloned strain of replicating murine type-C virus: association with induction of xenotropic-related RNA sequences contained in spleen focus-forming virus. *Virology* **85**:17–27.
 32. Troxler, D. H., E. Yuan, D. Linemeyer, S. Ruscetti, and E. M. Scolnick. 1978. Helper-independent mink cell focus-inducing strains of Friend murine type-C virus: potential relationship to the origin of replication-defective spleen focus-forming virus. *J. Exp. Med.* **148**:639–653.
 33. Wolff, L., R. Koller, and S. Ruscetti. 1982. Monoclonal antibody to spleen focus-forming virus-encoded gp52 provides a probe for the amino-terminal region of retroviral envelope proteins that confers dual tropism and xenotropism. *J. Virol.* **43**:472–481.