Evaluation of Feline Immunodeficiency Virus and Feline Leukemia Virus Transmembrane Peptides for Serological Diagnosis

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The general model for retrovirus transmembrane (TM) proteins proposed by Gallaher et al. (W. R. Gallaher, J. M. Ball, R. F. Garry, M. C. Griffin, and R. C. Montelaro, AIDS Res. Hum. Retroviruses 5:431–440, 1989) suggests that all retrovirus TM proteins may contain an immunodominant domain (Imd-TM peptide) located at the apex of the TM polypeptide. Although this Imd-TM peptide has been shown to be immunodominant in a variety of lentivirus infections, there has not been a detailed serological analysis of an oncovirus Imd-TM peptide as a diagnostic agent. We describe here an analysis of the antigenic properties and diagnostic potentials of the predicted Imd-TM peptides of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) in serological assays of sera from infected cats. The results of these studies demonstrate that antibodies specific to the Imd-TM peptide are detected within 2 weeks postinfection, are maintained at high levels for extended periods, and are not detectable in uninfected or FeLV-infected cats. In marked contrast, the FeLV Imd-TM peptide displayed only negligible levels of serological reactivity in FeLV-infected cats. These studies indicate that the peptide is a useful reagent for the detection of antibodies to FIV.

Domestic cats are subject to multiple retrovirus infections. Two of these infections, feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections, are associated with progressive immune deficiency diseases. FeLV is an exogenously replicating type C oncovirus which is spread horizontally and is estimated to infect up to 50% of free-roaming cats in the United States (9). The more recently isolated FIV is a member of the Lentivirinae subfamily of retroviruses and infects up to 14% of high-risk-group cats (17, 23). The frequency of FIV and FeLV coinfection is 16% in FeLV-infected cats in the United States (22). The prevalence of both oncovirus and lentivirus infections in cats creates a need for sensitive and specific diagnostic assays to differentiate between these viral infections, to accurately diagnose the infectious agent, and to elucidate possible cofactors of immune deficiency syndromes (18).

Gallaher et al. (7) have proposed a general model for the structure of retrovirus transmembrane (TM) proteins that indicates several characteristic structural motifs shared by oncoviruses and lentiviruses. One such peptide domain is located at the apex of the TM protein and is predicted to be an immunodominant protein segment of the TM molecule (Imd-TM peptide). The proposed Imd-TM domain has a characteristic loop structure stabilized by disulfide bonding between vicinal cysteines (see Fig. 1) that is also critical for peptide antigenicity. Thus, this model suggests that retrovirus Imd-TM peptides should be useful as specific diagnostic reagents in peptide-specific serological assays, including assays to detect FIV- and FeLV-specific antibodies.

The respective Imd-TM peptides of several lentiviruses have been exploited as diagnostic reagents in serological assays for human immunodeficiency virus types 1 and 2, simian immunodeficiency virus, and equine infectious anemia virus (1, 3, 8, 12, 19, 21). In each lentivirus system, the Imd-TM peptide displays a serological reactivity that develops rapidly to high titers after infection and that is maintained at high levels in long-term infections.

To date, the diagnostic potential of oncovirus Imd-TM peptides has not been evaluated in detail for any oncovirus system. Palker et al. (15) described an informative analysis of human T-cell leukemia virus I (HTLV-I) TM peptide antigenicity but did not assess the serological reactivity of the predicted HTLV-I Imd-TM peptide. Thus, the diagnostic potential of the oncovirus Imd-TM peptide remains to be determined. In this study, we have analyzed the antigenicity of the predicted FIV and FeLV Imd-TM peptides and evaluated their potential as diagnostic reagents in site-directed serology to distinguish between FIV and FeLV infections in cats.

We initially modeled the TM proteins of FIV Petaluma (Fig. 1A) (20) and FeLV (p15E) (Fig. 1B) and found that the structures predicted by Chou and Fasman (4, 5) are consistent with earlier models of retroviral TM proteins (3, 7). Synthetic peptides corresponding to the predicted FeLV and FIV Imd-TM domains (Fig. 1) were synthesized, purified, and characterized as previously described (6). These peptides were reacted in a solid-phase poly-L-lysine enzyme-linked immunosorbent assay (ELISA), which has been optimized for use with peptide antigens (2). The FIV and FeLV putative Imd-TM peptides were reacted with a panel of sera from 17 FIV-positive cats, 12 FeLV-feline AIDS transiently viremic "regressor" cats, 11 FeLV-feline AIDS persistently viremic "progressor" cats, and 4 uninfected cats (10). All serum samples in this reference panel were obtained from cats at least 100 days postinfection and thus represent long-term sera that should display a broad spectrum of virus-specific antibody responses.

The primary antibodies (cat sera) were diluted serially at 1/100, 1/1,000, and 1/10,000 in 10% BLOTTO (Carnation dry

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FIG. 1. TM protein sequences of FIV (A) and FeLV (B) modeled according to the rules of Chou and Fasman (4, 5) for secondary-structure prediction. Surface potential was predicted using the SurfacePlot algorithm of Parker et al. (16). Potential amphipathic alpha-helical regions were predicted using the Amphi algorithm of Margalit et al. (11). The results of these analyses were used to construct the proposed conformational model. The sequences of the predicted Imd-TM domains as proposed by Gallaher et al. (7) are shown in boxed areas. These Imd-TM peptides were synthesized for the serological assays described in the text.
FeLV "Immunodominant" Peptide

Potential glycosylation site

Random coil or unpredicted

Alpha helix

Beta sheet

Reverse turn

Hydrophobic amino acid

Polar uncharged amino acid

Charged amino acid

FIG. 1—Continued.
Reactivity of chronically infected cats to the homologous predicted Imd-TM domain peptides (pep). The titers are presented as ratios of test-cat serum reactivity to normal uninfected-cat serum reactivity to the respective peptides. 1.0, FeLV persistently viremic progressor (Pro.) cats; 2.0, FeLV transiently viremic regressor (Regr.) cats; and 3.0, FIV-infected cats. Serum samples were tested as described in the text.

FIG. 2. Reactivity of chronically infected cats to the homologous predicted Imd-TM domain peptides (pep). The titers are presented as ratios of test-cat serum reactivity to normal uninfected-cat serum reactivity to the respective peptides. 1.0, FeLV persistently viremic progressor (Pro.) cats; 2.0, FeLV transiently viremic regressor (Regr.) cats; and 3.0, FIV-infected cats. Serum samples were tested as described in the text.

readings of the 1/1,000 or the 1/10,000 dilution were used, the absorbance value was multiplied by the dilution factor of 10 or 100, respectively, before being divided by the normal-cat serum reactivity diluted at 1/100. Antibody reactivities to the FIV Imd-TM peptide fell into three general categories: 10 cats had relative reactivities in the 200 to 1,100 range, 4 cats had relative reactivities in the intermediate 50 to 200 range, and 3 cats had relative reactivities in the 10 to 50 range. There were no FIV-infected cats which had relative reactivity values of less than 10 with the FIV Imd-TM peptide. The levels of serological reactivity of the FIV Imd-TM peptide with cat sera are consistent with what has been described for other lentivirus infections, suggesting that this peptide may indeed provide a useful diagnostic reagent (1, 3, 8, 12).

The relative reactivities to the FeLV Imd-TM peptide in FeLV-infected cats are in distinct contrast to the high relative reactivities seen with FIV infections. The relative reactivities to the FeLV Imd-TM peptide in cats which experience transient viremia (regressor cats) were generally in the range of only 1.0 to 3.0 (Fig. 2). The relative reactivities of sera from persistently viremic cats (progressor cats) compared with specific-pathogen-free cats were generally below 1.0. The results with FeLV indicate that FeLV-infected cats make little or no antibody reactive with the FeLV peptide tested here. Thus, the predicted Imd-TM peptide of FeLV does not appear to be useful as a diagnostic reagent for FeLV-infected cats.

To examine further the potential of the FIV Imd-TM peptide as a diagnostic reagent, the kinetics of peptide-specific antibody responses in cats experimentally infected with FIV were examined. The serological data in Fig. 3 are presented as absorbance and not as the ratio of infected-cat serum reactivity to normal-cat serum reactivity. Figure 3 demonstrates that antibodies to the FIV Imd-TM peptide are detected early in FIV-infected cats; high titers are typically achieved within 2 weeks. In two cats (2531a and 2546a) infected by plasma transfer with 1,000 tissue culture infective doses of a field isolate of FIV, the antibody level was at least 200 times the normal cat reactivity to this peptide at some point between 3 and 6 weeks postinfection as reflected in the absorbance values obtained in peptide ELISA. Antibody titers in these two cats remained between 200 and 1,100 times normal cat levels for at least 18 weeks. Antibody levels in two cats (2104a and 2105a) infected with 10,000 tissue culture infective doses of FIV Petaluma from Crandel feline kidney cells increased very rapidly for the first 2 weeks, reached a plateau by 6 weeks, and remained between 50 and 200 times that of normal cats for at least 97 weeks. One cat (2528a) infected with 10,000 tissue culture infective doses of a field isolate of FIV grown in Crandel feline kidney cells experienced transient viremia (as determined by virus cultivation) and never developed detectable antibodies to the FIV Imd-TM peptide or to any FIV proteins (as determined by Western blot [immunoblot]), as was also the case with the control uninfected cat (2110a). This single FIV-inoculated cat was apparently able to clear the virus. Cats experimentally infected with FIV did not develop significant antibody reactivity to the FeLV Imd-TM peptide during the course of infection (data not shown). This finding is not surprising, considering the lack of sequence homology between the Imd-TM peptides of FIV and FeLV. Thus, antibodies to the FIV Imd-TM peptide are generated early and maintained for extended periods in FIV infections, and they are not detectable in uninfected cats.

To investigate whether antibodies to the FeLV Imd-TM peptide might be detectable early postinfection, longitudinal
serum samples from cats experimentally infected with FeLV were examined in standard ELISAs. The results of these serological assays demonstrated a lack of significant antibody responses to the FeLV Imd-TM peptide during the first 3 months postinfection in both transiently and persistently viremic FeLV-infected cats (data not shown). These results confirm that the feline oncovirus and lentivirus TM proteins differ markedly in their immunogenicities during the early stages of infection.

The basis for the differential antigenicity of FIV and FeLV Imd-TM peptide domains is not certain. Our structural models (Fig. 1) for the FIV-TM Petaluma and FeLV-TM (p15E) proteins are consistent with the general model presented earlier (7). The observed strong antigenicity of the FIV Imd-TM peptide and the lack of antigenicity of the FeLV Imd-TM peptide are in agreement with the respective antigenic potentials predicted by the SurfacePlot algorithm (16). Both the TM model and the SurfacePlot algorithm accurately predict a high antigenic potential for the FIV Imd-TM peptide. However, for human immunodeficiency virus type 1, simian immunodeficiency virus, and equine infectious anemia virus Imd-TM peptides the predictive schemes were in conflict with SurfacePlot, failing to predict that these peptide domains would constitute predominant epitopes. Clearly, sequence considerations alone can not reliably predict peptide-specific immunoreactivities, which must be determined experimentally.

In conclusion, these studies demonstrate that the predicted FIV Imd-TM peptide satisfies the criteria for utilization in site-directed serology, making it an excellent candidate for the development of a commercial diagnostic test (13, 14). The FIV Imd-TM peptide is conserved in all sequenced isolates found in GenBank to date, and therefore a test based on this peptide can potentially have worldwide application.

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