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Identification of a Linear Neutralization Site within the Third Variable Region of the Feline Immunodeficiency Virus Envelope

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Synthetic peptides have been used to map linear B-cell epitopes of the third variable (V3) region of the feline immunodeficiency virus (FIV) external membrane glycoprotein gp120. The analysis of sera from naturally and experimentally FIV-infected cats by Pepscan and enzyme immunoassay with four partially overlapping peptides evidenced three antibody-binding domains, two of which mapped in the carboxyl-terminal half of V3. In particular, the V3.3 sequence (Gly-392–Phe-413) turned out to be important for in vitro neutralization of the virus in that the peptide inhibited the FIV-neutralizing activity of pooled immune cat sera, and on the other hand, cat sera raised against this peptide effectively neutralized FIV infectivity for Crandell feline kidney cells.

Feline immunodeficiency virus (FIV) is a lentivirus first isolated from immunodeficient domestic cats in California in 1986 (36) and subsequently evidenced in cats throughout the world (3, 48). As it has many of the biological and pathogenic features of human immunodeficiency virus (HIV), it is considered a valuable tool for investigating the pathogenesis of human AIDS and strategies for the preparation of anti-lentiviral drugs and vaccines (35, 40).

As in HIV and other lentiviruses, the *env* gene in FIV exhibits a considerable degree of diversity among different isolates, clustered in discrete segments of its sequence known as variable regions. Eight or possibly nine such variable regions have been identified in FIV (30, 34, 37, 41). Although alignment of amino acid sequences shows that the HIV type 1 (HIV-1) and FIV *env* proteins are distantly related, with only about 5% overall identity, analogies exist in where the variable and constant regions are located within the external envelope glycoproteins of the two viruses. This has led to speculation that the spatial folding and overall configuration of these molecules might be conserved in the two viruses (34).

The immune responses to FIV and the viral antigens involved are still poorly characterized. For example, neutralizing antibody (NA) assays based on inhibition of FIV infectivity for the Crandell feline kidney fibroblast (CrFK) cell line have shown that infected cats develop broadly reactive NA, as shown by the presence of NA in infected cats from different geographical areas and by the ability of NA to neutralize different FIV isolates (45; unpublished results), but whether FIV also induces type-specific NA has yet to be established.

The third variable (V3) region of the HIV-1 external glycoprotein contains important determinants of cell tropism, cytopathicity, and infectivity (5, 6, 10, 16, 20) and prominent immunoreactive domains which are involved in both type-restricted and broadly reactive neutralization (15, 21–23, 31, 32). Similar observations have been made for other lentiviruses (2, 24). As the V3 regions of FIV and HIV-1 have a number of analogous structures (34), including similar distances from the carboxyl terminus of the molecule, the presence of a Cys-Cys bond, and a computer-predicted β-turn and glycosylation site, we reasoned that FIV V3 might also contain immunologically important domains. Here, synthetic peptides were used to investigate the antibody-reactive domains present in FIV V3 and whether such domains are involved in virus neutralization.

MATERIALS AND METHODS

Viruses. Three different FIV isolates were used. The California isolate Petaluma (FIV-P) and the Dutch isolate Amsterdam 6 (FIV-A6) were propagated in persistently infected CrFK cells as described previously (36). The Pisa-M2 isolate (FIV-M2), originally obtained from a clinically healthy feline leukemia virus-negative cat and freed of the feline syncytium-forming spumavirus which was initially present by two consecutive passages of cell-free plasma, was propagated in vivo by monthly passages in specific-pathogen-free (SPF) cats (IFFA Credo, Como, Italy). So far, we have been unable to adapt FIV-M2 to CrFK cells. For enzyme-linked immunosorbent assays (ELISAs; see below), virus was concentrated from tissue culture supernatants by ultrafiltration (Minitan; Millipore) and purified by density gradient centrifugation (29). Purified FIV was disrupted in phosphate-buffered saline (PBS) containing 0.25% sodium dodecyl sulfate (SDS) at 4°C with gentle stirring for 2 h.

Synthetic peptides. From the published sequence of the 34TF10 clone of FIV-P (37), four partially overlapping 22- or 23-amino-acid peptides covering the entire variable V3 domain of the FIV *env* region were manually synthesized with *N*-α-fluorenylmethoxycarbonyl (FMOC)-protected amino acids and a *p*-alkoxybenzyl alcohol resin as the solid phase as described by Atherton et al. (1). The purity of all peptide preparations, assessed by high-pressure liquid chromatography, exceeded 90%. The sequences of the four peptides (V3.1, V3.2, V3.3, and V3.4) are shown in Fig. 1. Pepscan

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V3.1 $_{373}$CKWEEAKVKFHCQRTQSQPGSW$_{394}$

V3.2 $_{382}$FHCQRTQSQPGSWFRAISSWKQ$_{403}$

V3.3 $_{392}$GSWFRAISSWKQRNRWEWRPDF$_{413}$

V3.4 $_{402}$KQRNRWEWRPDFKSKKVKISLPC$_{424}$

FIG. 1. Amino acid sequences (one-letter code) of the four synthetic peptides spanning residues 373 to 424 of the deduced *env* V3 protein domain (37). Underlined letters indicate variable amino acid positions.

analysis of the V3 domain required the synthesis of 46 hexapeptides, overlapping one another by five amino acids, spanning the *env* region from Lys-374 to Cys-424. The synthesis was performed as described by Geysen et al. (13), by using FMOC chemistry and polyethylene pins as the solid phase, following the manufacturer's recommendations (CRB, Cambridge, United Kingdom).

Cat sera. A panel of 77 cat sera were used for measuring antibody reactivity against the synthetic peptides. The panel included 48 sera from field cats, 20 sera from noninfected SPF cats, and 9 sera from SPF cats experimentally infected with FIV-M2. The infectious dose used (2 ml of freshly collected blood) leads to seroconversion in 4 to 6 weeks and to a reduction in the number of circulating $CD4^+$ lymphocytes by almost two-thirds in 1 year. Infected SPF animals have remained symptom free throughout the period of observation. All sera were tested with a commercial kit (Cite Combo FIV-FeLV; Agritec Systems, Portland, Maine) and by Western immunoblot on infected culture lysates (38) and/or by neutralization assay (45). Sera were considered FIV antibody positive when they reacted in at least two assays.

Immunization of cats with synthetic peptides. Eight-month-old SPF cats were immunized subcutaneously with the peptides (total volume, 1 ml) according to the following schedule: 1 mg of peptide in Freund's complete adjuvant (1:1) on day 0, and 500 μg of peptide in Freund's incomplete adjuvant on days 15, 30, 45, and 60. Blood samples were collected under Ketalar (Inoketam 500; Virbac) anesthesia 7 days after each inoculation.

ELISA for antipeptide antibodies. For the standard ELISA, 96-well microtiter plates (Maxisorb type; Nunc) were coated overnight with the peptides at 10 μg/ml in 0.1 M carbonate buffer, pH 9.8. The wells were washed four times with PBS containing 0.05% Tween 20 (PBS-Tw) and postcoated with 20 μg of bovine serum albumin (BSA; Serva) per ml in PBS. Sera diluted in PBS supplemented with 1% Tween 20, 0.1% BSA, and 0.05% sodium azide were incubated in the wells for 2 h. After the wells were washed, a peroxidase-conjugated goat antiserum directed against cat total immunoglobulin (Ig) or IgG (Chemicon, Temecula, Calif.), diluted in PBS-Tw containing 20 μg of BSA per ml was added to the wells. After 1 h, the plates were washed, and the presence of bound enzyme-labeled antibody was revealed by a chromogenic substrate (*o*-phenylene diamine; Calbiochem, La Jolla, Calif.). The optical density at 492 nm (OD_{492}) was read in an automatic ELISA plate reader (Multiscan; Flow). All steps were performed at room temperature (RT); postcoating was done with 150 μl of BSA in PBS per well; peptides, sera, conjugate, and substrate were added in volumes of 100 μl per well. For the Pepscan ELISA, sera were reacted against the whole series of pin-bound hexapeptides according to the manufacturer's specifications with minor modifications. Reactivity to the single peptides was expressed as (sample OD − control OD)/control OD, where the control OD was the value given by a pool of six seronegative cat sera. A peptide was considered highly reactive with a cat serum when its reactivity value ranked among the highest 15, medium reactive when it ranked 16 to 30, and low reactive when it ranked 31 to 46.

ELISA for anti-FIV antibody. ELISA plates were coated overnight with 1 μg of SDS-disrupted virus in 100 μl of carbonate buffer (pH 9.6) per well. The plates were subsequently postcoated with 150 μl of PBS containing 1% BSA for 1 h at RT. Samples (100 μl) were added to the plates and incubated for 1 h at RT. Bound IgG antibodies were revealed as described above.

FIV NA assay. Sera were tested for NA in a recently described assay based on inhibition of FIV-induced syncytium formation (45). Briefly, twofold dilutions of heat-inactivated serum were mixed with 100 syncytium-forming units of FIV-P and, in some experiments, FIV-A6 in 24-well plates. After 1 h at room temperature, 1 ml of medium containing 2×10^4 CrFK cells adapted to grow in 0.5% fetal bovine serum was added to each well. Six days later, the cultures were stained, and syncytia were counted under a microscope.

Flow cytometry. Flow cytometry analysis of antibody-binding FL4 cells persistently infected with FIV-P (47) was performed with an Epics Elite flow cytometer (Coulter Electronics, Hialeah, Fla.). Cells (10^5) were incubated for 1 h with an appropriate dilution of cat serum in PBS containing 3% fetal bovine serum and 0.1% sodium azide. After being washed, the cells were incubated for 30 min with a fluorescein-conjugated goat anti-cat IgG (Sigma, St. Louis, Mo.) and, after further washing, fixed in PBS containing 1% *p*-formaldehyde, 2% glucose, and 0.1% sodium azide. All steps were performed at 4°C. Standard alignment with fluorescent microspheres was done prior to data acquisition. A 525-nm bandpass filter was used for detection of green fluorescence. The data were first collected in a two-parameter histogram of size versus granularity. After electronic removal of any debris from the analysis by selective gating, the data were transferred to a single-parameter log fluorescence histogram. Data were collected from approximately 10,000 cells for each experimental condition.

Peptide inhibition of FIV neutralization. For evaluating the ability of the V3-based peptides to inhibit the neutralizing activity of anti-FIV cat serum, an appropriate dilution of pooled neutralizing cat sera was mixed with several concentrations of each peptide before being mixed with the virus. The mixtures were then processed as for the NA assay. The number of syncytia per well produced in the presence of the peptides was compared with the number of syncytia obtained when the virus had been preincubated with the neutralizing antiserum alone.

Antigenicity prediction. The positions of possible antigenic determinants on the synthetic peptides were predicted by calculating hydrophilicity plots by the method of Hopp and Woods (18) with the PC/Gene computer program (Intelligenetics).

RESULTS

Pepscan analysis. Pepscan analysis of the V3 region with sera from 10 randomly selected, naturally infected cats

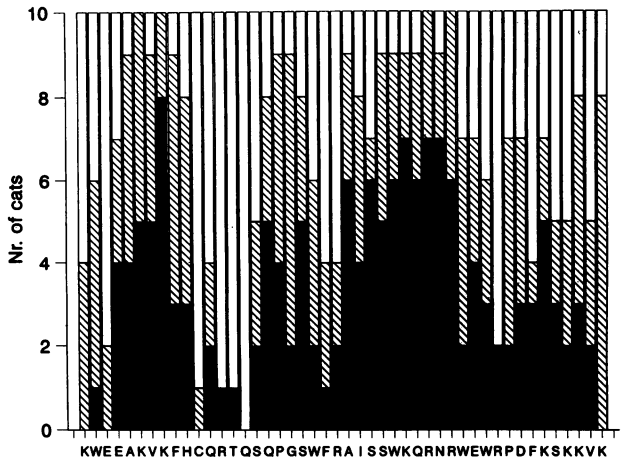

FIG. 2. Immunoreactivity of cat sera with 46 Pepscan hexapeptides covering the *env* V3 domain. Bars indicate the reaction intensity in ELISA (solid, high; hatched, medium; open, low), and the height of each bar corresponds to the number of cats displaying the corresponding degree of reactivity. The letters at the base of each bar show the first amino acid of the hexapeptide, so that the sequence of each hexapeptide can be easily read (KWEEAK is the first, WEEAKV is the second, and so on). The sequence of the last hexapeptide is KISLPC.

revealed several antibody-binding domains. Although individual sera varied considerably in their ability to bind support-coupled hexapeptides (data not shown), there were three domains which reacted with at least 9 of the 10 sera (Fig. 2). This result indicated that the V3 region contains conserved linear B-cell epitopes that are recognized by sera from a high proportion of naturally infected cats and was therefore worth further investigation.

Reactivity of cat sera to synthetic peptides covering the V3 region. Sera from nine SPF cats experimentally infected with FIV-M2 25 to 50 weeks earlier were screened by ELISA for Ig reactivity to the four partially overlapping synthetic peptides V3.1, V3.2, V3.3, and V3.4, spanning the entire V3 region. The results are summarized in Fig. 3A. High antibody prevalences and levels were observed with peptides V3.3 and V3.4, while peptide V3.2 gave the lowest readings. Background noise given by sera from control uninfected SPF cats was usually low.

Sera from naturally infected field cats displayed a similar pattern of reactivity, the highest levels and prevalences of antibodies again being recorded against V3.3 and V3.4 and the lowest against V3.2 (Fig. 3B). As expected (19), background noise given by sera from seronegative field cats was higher than that by sera from uninfected SPF cats. Nonetheless, the differences between the mean reactivities of sera from FIV-seropositive and seronegative cats were highly significant for V3.1 ($t = 5.86$; $P < 10^{-4}$), V3.3 ($t = 8.14$; $P < 10^{-6}$), and V3.4 ($t = 9.34$; $P < 10^{-6}$), as assessed by Student's t test, calculated by using the normally distributed natural log of the OD. These results confirmed the presence of linear antibody-binding domains within the V3 region.

The low reactivity observed with peptide V3.2 could be ascribed to a relatively low affinity for the plates or to a masking of antigenic determinants once the peptide is bound to the solid phase. When V3.2 was probed in an ELISA with the homologous antiserum raised in a cat, a titer of 1:3,200 was obtained. This reactivity, although lower than those recorded with the other peptide-homologous antiserum combinations (titers, ≥1:32,000), was obviously higher than the readings obtained with preimmune sera (titers, <1:100). These findings indicate that V3.2 actually bound to the plates and maintained some reactivity. The fact that FIV-seropositive cats could not be discriminated from seronegative cats on the basis of anti-V3.2 reactivity could depend on an intrinsic lower antigenicity of this peptide, as also indicated by the hydrophilicity plot of the four peptides (Fig. 4).

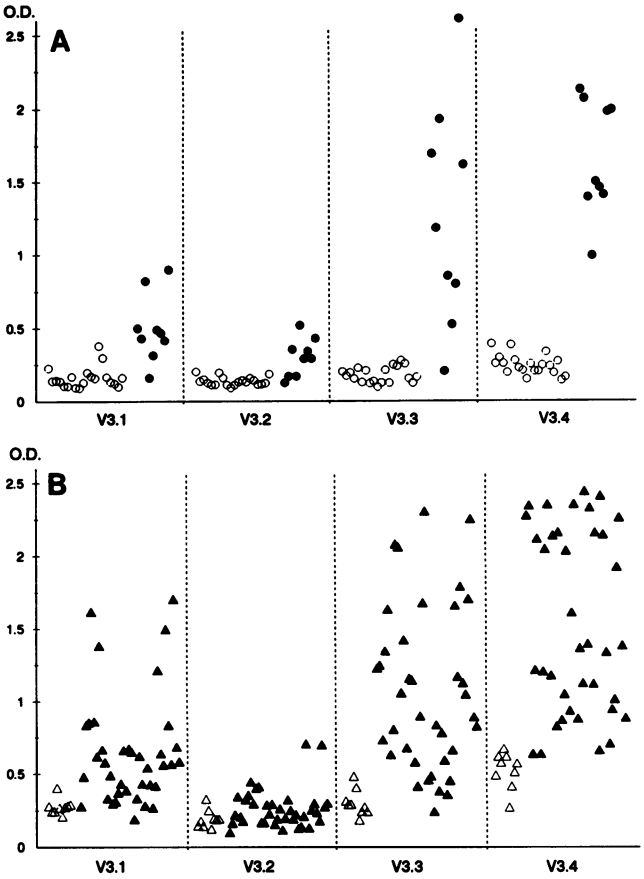

FIG. 3. Antibodies to the V3 synthetic peptides in FIV-infected cats. The reactivity of cat sera, diluted 1:500, with synthetic peptides V3.1, V3.2, V3.3, and V3.4 was determined by ELISA and expressed as optical density (OD). (A) Open circles, sera from uninfected SPF cats; solid circles, sera from SPF cats experimentally infected 25 to 50 weeks previously. (B) Open and solid triangles refer to serum samples from serologically FIV-negative and FIV-positive field cats, respectively.

Antipeptide antibody responses in infected and peptide-immunized cats. The development of antibodies to the V3-based peptides during FIV infection was studied by testing sera collected at various intervals from FIV-M2-infected SPF cats against the four peptides. As shown in Fig. 5, antibodies to peptides V3.1, V3.3, and V3.4 appeared at 3 to 4 weeks postinfection and increased in level in subsequent weeks. No antibodies to the V3.2 peptide were detected during the period of observation.

To extend these findings, we assayed sequential serum samples obtained from SPF cats immunized with the four synthetic peptides. Samples were collected 7 days after each inoculation and tested for antipeptide IgG by ELISA. All peptides inoculated in unconjugated form with complete and incomplete Freund's adjuvant elicited an antipeptide anti-

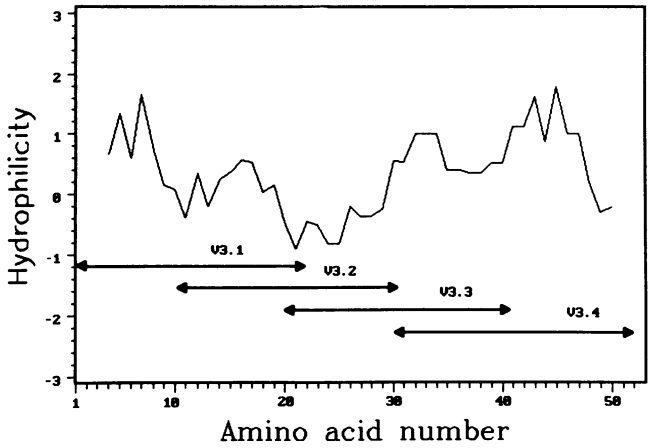

FIG. 4. Hydrophilicity profile of the V3 sequence, computed for an average group length of six amino acids. The vertical axis shows units of hydrophilicity, which are based on the charges of the amino acid side chains. The horizontal axis shows the amino acid position in the peptide sequence.

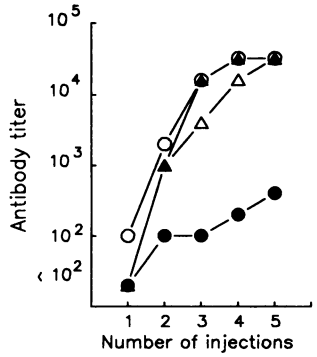

FIG. 6. Antipeptide antibodies in immunized cats. Anti-V3.1 (○), -V3.2 (●), -V3.3 (△), or -V3.4 (▲) antibody titer was assayed by ELISA in cat sera collected 7 days after immunization. Serum titers are expressed as the highest dilution giving OD readings greater than the mean + 3 standard deviations for three wells receiving preimmune cat serum.

body response (Fig. 6). Antibodies to the V3.1, V3.3, and V3.4 peptides appeared after the second inoculation and reached high levels after further boosts. The V3.2 peptide induced a weak antibody response even after five antigen injections. The low immunogenicity of this peptide was confirmed by the observation that mice immunized with it responded poorly compared with mice immunized with the other peptides (data not shown).

Taken together, these results confirmed that the V3 region of FIV and especially its carboxyl-terminal half contains B-cell epitopes that are highly immunogenic and elicit prompt antibody responses both after viral infection and after inoculation of synthetic peptides.

Reactivity of antipeptide sera with whole virus and virus-infected cells. In these experiments, we examined the reactivity of cat antipeptide sera by ELISA on purified FIV-P and by flow cytometry on infected cells. Only the antiserum to V3.3 recognized purified whole virus effectively, yielding a titer of 1:3,200, whereas the other antisera were nonreactive. Accordingly, the flow cytometry assays revealed that

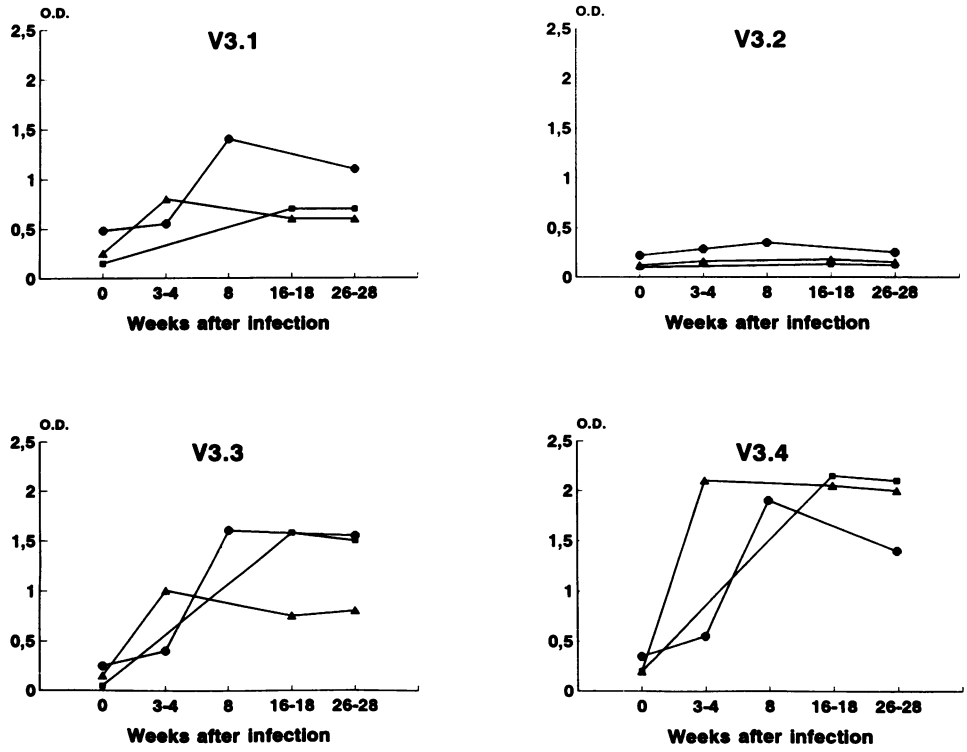

FIG. 5. Kinetics of antibody production to the V3 synthetic peptides in SPF cats experimentally infected with FIV-M2. Reactivity in ELISA of cat sera diluted 1:500 with peptides V3.1, V3.2, V3.3, and V3.4 is expressed as optical density (OD). Different symbols are used for sera from individual cats.

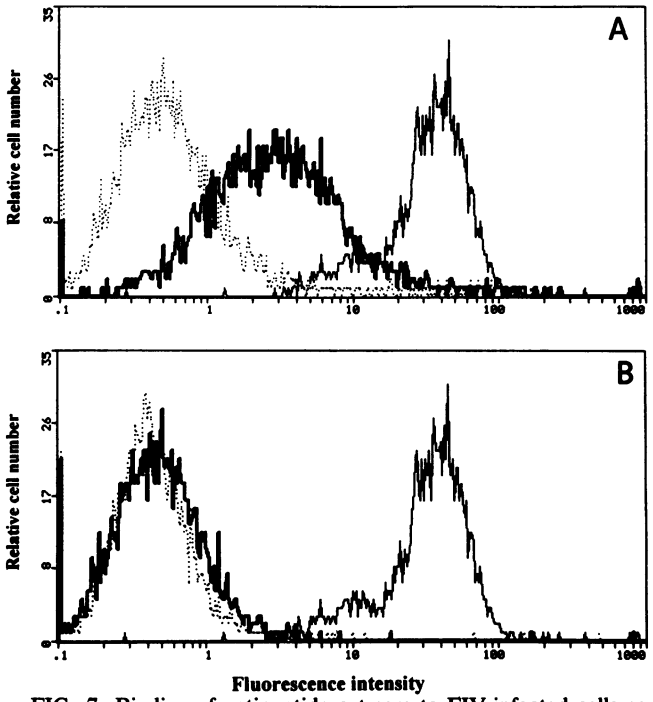

FIG. 7. Binding of antipeptide cat sera to FIV-infected cells as assessed by flow cytometry. (A) FL4 cells persistently infected with FIV-P were incubated with anti-V3.3 serum (——), preimmune serum (.....), or a high-titer serum obtained from an SPF cat infected with FIV-M2 (——) for 30 min on ice. The cells were then washed, incubated with goat anti-cat IgG coupled with fluorescein isothiocyanate for 30 min, washed again, fixed with *p*-formaldehyde, and analyzed for fluorescence intensity. (B) Results obtained in similar experiments with the anti-V3.4 (——) serum. This panel is also representative of results obtained with antisera to peptides V3.1 and V3.2.

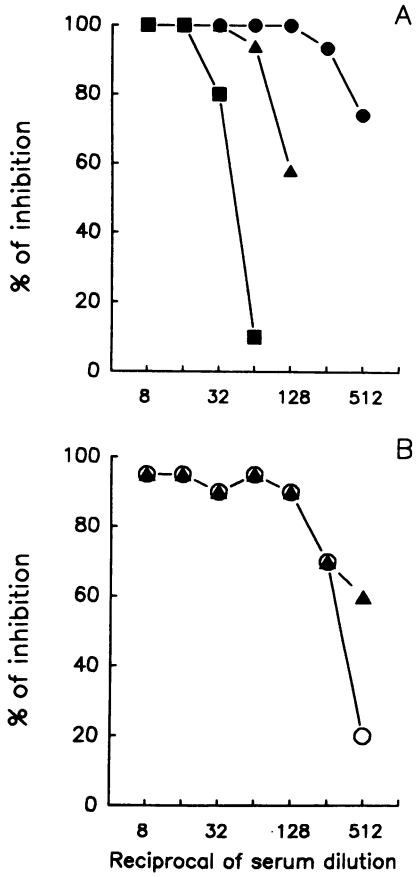

FIG. 8. FIV neutralizing activity of cat anti-V3.3 antibody. (A) Immune sera collected from three SPF cats following five injections of V3.3 peptide were tested for neutralizing activity as described in Materials and Methods with FIV-P. Each symbol represents an individual cat serum. Preimmune cat serum had no detectable NA (data not shown). (B) Immune serum collected after 4 (○) and 5 (▲) V3.3 injections was tested for NA with FIV-A6. Preimmune cat serum and anti-V3.3 cat sera obtained after one and two peptide injections had no NA (data not shown).

the antiserum to V3.3, diluted 1:20, stained a high proportion (63%) of persistently infected FL4 cells, whereas the antisera to the other peptides showed the same staining pattern as the preimmune serum (Fig. 7A and B). Thus, these results clearly indicated that the V3.3 peptide evokes antibodies against a viral epitope which is exposed on the surface of the FIV virion and of virus-infected cells.

FIV neutralization by antipeptide sera. To address the possibility that the immunoreactive domains identified in the previous experiments were involved in virus neutralization, peptide-specific immune cat sera were tested for their capacity to neutralize the infectivity of FIV-P for CrFK cells. The neutralization studies were performed several times on coded samples, including preimmune serum and unrelated antisera as controls. In the results shown in Fig. 8A, three anti-V3.3 sera collected after five peptide injections consistently produced 100% inhibition of FIV-induced syncytium formation. In particular, one serum completely prevented the formation of virus-induced syncytia up to a dilution of 1:128. The antisera to the other peptides, as well as preimmune serum, were consistently negative (lowest dilution tested, 1:4).

As already mentioned, the neutralization assay used in these experiments detects broadly reactive NA. Apparently this also held for the neutralizing activity of the anti-V3.3 antibodies, which efficiently neutralized another FIV isolate, FIV-A6. As shown in Fig. 8B, anti-V3.3 sera collected after four and five antigen injections inhibited approximately 90 to 95% of syncytium production by FIV-A6.

Inhibition of neutralizing activity of sera from FIV-infected cats by V3 peptides. The previous results suggested that the domain represented by the synthetic peptide V3.3 is involved in FIV neutralization and that the respective NAs involved are broadly reactive. To investigate whether this neutralization site plays a role in FIV neutralization by antibodies produced during infection, we examined the four V3 peptides for their ability to inhibit the neutralizing activity of sera from infected cats. The experiments were possible because at concentrations of between 5 and 100 μg/ml, the synthetic peptides did not affect syncytium formation by FIV (data not shown). Pooled sera obtained from SPF cats infected with FIV-M2 were diluted 1:2,000 and incubated with the peptides at RT for 1 h. The mixtures were then processed as for the standard neutralization assay except that the numbers of syncytium-forming units of FIV-P were kept low. As shown in Table 1, peptide V3.3 consistently inhibited the ability of serum to neutralize FIV infectivity. In contrast, peptides V3.1, V3.2, and V3.4 had no effect. Although inhibition of neutralization by V3.3 was

TABLE 1. Inhibition of the neutralizing activity of anti-FIV serum by peptide V3.3[a]

Expt no.	No. of syncytia (mean ± SD) produced in the presence of:			
	Normal cat serum (1:1,000)	V3.3 + normal cat serum (1:1,000)	Immune serum (1:2,000)	V3.3 + immune serum (1:2,000)
1	38 ± 3.6 (100%)	32 ± 2.1 (84%)	17 ± 2.6 (45%)	30 ± 3.5 (79%)
2	24 ± 2.5 (100%)	26 ± 4.0 (108%)	6 ± 2.0 (25%)	19 ± 4.3 (79%)

[a] Peptide V3.3 was used at a final concentration of 40 μg/ml. Immune serum was a pool of three sera from SPF cats infected with FIV-M2 8 months earlier and neutralized 100% of FIV-induced syncytia up to a dilution of 1:500. Data are expressed as mean number of syncytia per well for triplicate cultures. The percentage of the number of syncytia is given in parentheses.

dose dependent within the 10- to 40-μg range, complete inhibition of the neutralizing activity of serum was never achieved (data not shown).

DISCUSSION

Pepscan analysis of the entire length of the FIV V3 amino acid sequence showed that this region contains linear domains recognized by sera from naturally FIV-infected cats. ELISA studies probing a larger number of cat sera against four partially overlapping, 22- or 23-residue-long peptides covering the entire V3 sequence showed that the carboxyl-terminal half of the V3 region, represented by peptides V3.3 and V3.4, contains one or more conserved immunodominant domains. The V3.1 sequence also seems to be immunogenic for cats, in that one immunodominant domain was detected in this segment by Pepscan analysis and 55% of naturally or experimentally infected cats evidenced antibodies to the peptide by ELISA. In contrast, peptide V3.2 was not recognized by the great majority of FIV-positive cat sera, suggesting that this sequence of V3 does not harbor B-cell epitopes that are perceived as such during infection. It should be noted, however, that the results of Pepscan analysis and the synthetic-peptide ELISA were slightly different; this was particularly true for the sequence covered by peptide V3.2, which was recognized more frequently in the Pepscan. This discordance is probably due to differences in techniques or to conformational differences (7).

All of the SPF cats immunized with the four synthetic peptides developed antibodies which reacted in the ELISA with the corresponding peptide, confirming previous findings that peptides may evoke strong antibody responses even without being linked to a carrier protein (33). This reactivity was probably due to the presence of B- and T-cell epitopes on each peptide. That the four V3-based peptides used in these experiments contain T-cell epitopes was shown by their ability to elicit a delayed-type hypersensitivity reaction when injected intradermally into FIV-infected cats (results not shown). Consistent with the results obtained with infected cat sera, the lowest levels of antipeptide antibodies were produced after immunization with peptide V3.2. This confirms that the gp120 section covered by peptide V3.2 appears to be inherently poorly immunogenic, as also predicted by computer analysis of its amino acid sequence.

Of the antipeptide sera raised in cats, only anti-V3.3 reacted in the ELISA with purified FIV and stained FIV-infected cells. This implies that the V3 portion of gp120 contains an antibody-binding site which is also accessible to antibody on the native molecule, as found in the intact virus envelope. In HIV-1 gp120, the accessibility of immunoreactive domains to Ig has been shown to be affected by the folding of the molecule as well as by its glycosylation (9, 17, 28). Interestingly, the anti-V3.3 serum was the only one that inhibited FIV infectivity for CrFK cells, showing that the linear immunoreactive domain(s) encompassed by peptide V3.3 is not only exposed on the virion surface but also involved in virus neutralization in vitro. This conclusion was strengthened by the observation that only this peptide could competitively interfere with FIV neutralization by infected-cat sera.

It should be pointed out that, although the sequences of the peptides used in this study were deduced from the published sequence of FIV-P, the anti-V3.3 serum neutralized both FIV-P and FIV-A6 with similar potency. This confirms that our neutralization assay detects cross-reacting NA (45) and suggests that the neutralizing site involved is conserved in distant isolates and recognized by NA present in most infected cats. The finding that peptide V3.3 inhibited FIV-P neutralization by sera from cats experimentally infected with FIV-M2 (this isolate could not be used in neutralization because it does not grow on CrFK cells) confirms this possibility. Broadly neutralizing antibodies to HIV-1 have been obtained by immunization of mice with V3 peptides (46).

In HIV-1 gp120, important neutralization epitopes have been identified in the V3 region as well as in the receptor-binding domain and other regions (11, 23, 25, 31, 42–44). Initially, NA to HIV-1 V3, which is generally known as the principal neutralizing domain of the virus, were described as specific for individual or related isolates, but recently monoclonal and polyclonal anti-V3 antibodies which neutralize divergent HIV-1 isolates have been described (15, 22, 32, 46). Thus, the presence in FIV V3 of domains which evoke broadly reactive NA is not surprising, especially when one considers that amino acid sequence variability in this region is considerably lower among FIV than among HIV-1 isolates (34, 39). According to published sequences, the V3.3 segments of FIV-A6 and FIV-M2 differ from that of FIV-P by four and five amino acids, respectively, of which two and three, respectively, are conservative changes (39).

Many aspects of FIV biology are still poorly understood. For example, the receptor-binding domain and other regions critical for infectivity have not yet been identified. This initial characterization of an FIV gp120 region which elicits broadly reactive cat NA may provide a starting point. Further studies will be required to better define the immunologically relevant domains of the FIV envelope. Results not reported here have shown that synthetic peptides corresponding to other variable regions of FIV gp120 V4 (Gly-474–Asn-488), V5 (Cys-541–Gly-575), and V6 (Pro-600–Lys-617) were recognized by variable percentages of sera from infected cats but did not induce NA when injected into cats. Similar studies with the remaining regions of the gp120 molecule and analysis of monoclonal antibodies will most likely lead to recognition of additional linear and conformational neutralizing epitopes.

Conformational epitopes in V3, along with epitopes lying outside the V3 region, play important roles in HIV-1 neutralization (11, 25, 42, 44). That additional neutralizing determinants exist in FIV is suggested by the failure of peptide V3.3 to completely block the neutralizing activity of infected-cat sera. It is becoming increasingly evident that humoral responses play crucial roles in protective immunity against HIV-1 infection and its progression (4, 8, 14) and that the V3 region is an important determinant to include in candidate HIV-1 vaccines (27). Recently, a correlation has also been observed between NA titer and protection conferred by experimental FIV vaccines (49). Here we have shown that carrier-free single-peptide immunization can evoke broadly reacting NA to FIV. To design anti-FIV immunization strategies, it is therefore important to study whether anti-V3.3 antibodies may play a role in protection against FIV. This can now be done by active or passive immunization of cats and subsequent challenge with FIV. In preliminary experiments, dilutions of anti-V3.3 which effectively neutralized FIV infectivity for CrFK cells were unable to block FIV infectivity for a lymphoid T-cell line. As lentiviruses may be neutralized by different mechanisms depending on the cell type (26), the possibility exists that the NA evidenced in CrFK cells neutralizes FIV infectivity for certain cell types but not for others. This would considerably diminish their protective value.

The simian immunodeficiency virus and FIV are presently considered valuable models for studying aspects of AIDS pathogenesis and therapy that cannot be easily approached in humans (12, 40). The V3 region of simian immunodeficiency virus harbors neutralizing epitopes, but these appear to be conformationally determined (24). The present findings, showing that the V3 region of FIV contains linear neutralizing epitopes, suggest but do not definitely prove that this region in FIV is functionally similar to the V3 loop in HIV-1 and thus enhance the value of FIV as an animal model for AIDS studies.

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