

## Antibody Response in Cats to the Envelope Proteins of Feline Immunodeficiency Virus: Identification of an Immunodominant Neutralization Domain

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Received July 6, 1993; accepted September 21, 1993

Overlapping fragments of the envelope protein of feline immunodeficiency virus (FIV) have been expressed in *Escherichia coli*. Screening of cat sera for antibodies to these fragments revealed that the immunodominant domain of the FIV envelope is localized within the transmembrane protein (amino acids 687-741) and that both the variable region 3 (SU3, aa 385-417) and the COOH-terminus (aa 599-611) of the surface protein (SU) are highly immunogenic. Of all rabbit sera raised to the envelope protein fragments only the serum directed to SU3 was neutralizing. Both FIV-infected and SU3-immunized cats elicited neutralizing antibodies to SU3. Neutralizing antibodies in sera of infected cats could be absorbed by SU3, showing that SU3 is a major neutralization domain of FIV. © 1994 Academic Press, Inc.

### INTRODUCTION

The feline immunodeficiency virus (FIV), a lentivirus, is a major pathogen causing AIDS in cats (Pedersen *et al.*, 1987; Torten *et al.*, 1991; Barlough *et al.*, 1991). With respect to infection dynamics, host response and course of disease, FIV infection of cats is similar to HIV-1 infection of man. FIV vaccines should prevent cats from getting AIDS; their study can contribute to the development of a vaccine against HIV-1 infection. An efficacious FIV vaccine probably requires the induction of cellular as well as humoral immunity. Humoral immunity encompasses the production of antibodies which induce, e.g., antibody-dependent cell-mediated cytotoxicity, virus neutralization, but also enhancement of infection (Robinson and Mitchell, 1991).

In an effort to characterize the natural humoral response against FIV we have focused on its envelope proteins. The FIV envelope gene encodes a precursor protein consisting of the 20-kDa leader (L), the surface (SU) and the transmembrane (TM) proteins. Only the SU and TM protein become incorporated within the viral particle (Verschoor *et al.*, 1993). Therefore, our study has been confined to the SU and TM proteins which are expected to contain the biologically most important antigenic sites of FIV. To map antigenic sites on the FIV SU and TM protein, we screened cat sera for the presence of antibodies against fragments of the SU and TM protein expressed in *Escherichia coli*. In such

fragments the conformation of the native intact proteins as synthesized in eukaryotic cells is lost and primarily antibodies directed to linear B-cell epitopes will be detected. However, like conformational B-cell epitopes linear B-cell epitopes are capable of eliciting enhancing and neutralizing antibodies as evidenced, e.g., for the V3-loop on the envelope surface protein of HIV-1 (Palker *et al.*, 1988; Goudsmit *et al.*, 1988; Rusche *et al.*, 1988).

In addition to mapping antigenic sites on the envelope proteins of FIV we have attempted to assign biological activity to antibodies elicited against defined envelope protein fragments. As will be shown, the SU protein of FIV contains an immunodominant domain which is able to elicit neutralizing antibodies in cats and rabbits.

### MATERIALS AND METHODS

#### Plasmids encoding fusion proteins

The pOTSKF33 plasmid vector (Chiang *et al.*, 1989; Krone *et al.*, 1988) encoding the NH<sub>2</sub>-terminal 6-kDa part of galactokinase (galK) controlled by an inducible promoter was used to construct fusion proteins between galK and the SU protein of FIV strain UT113 [EMBL Accession No. X60725 (Verschoor *et al.*, 1993)]. Standard cloning techniques (Sambrook *et al.*, 1989) using convenient restriction enzyme sites within the FIV-UT113 *env* gene were applied to obtain the galK-SU-*env* in frame fusion constructs (Fig. 1). The polymerase chain reaction (PCR) was used to construct the galK-SU-*env* fusion encoding the NH<sub>2</sub>-termi-

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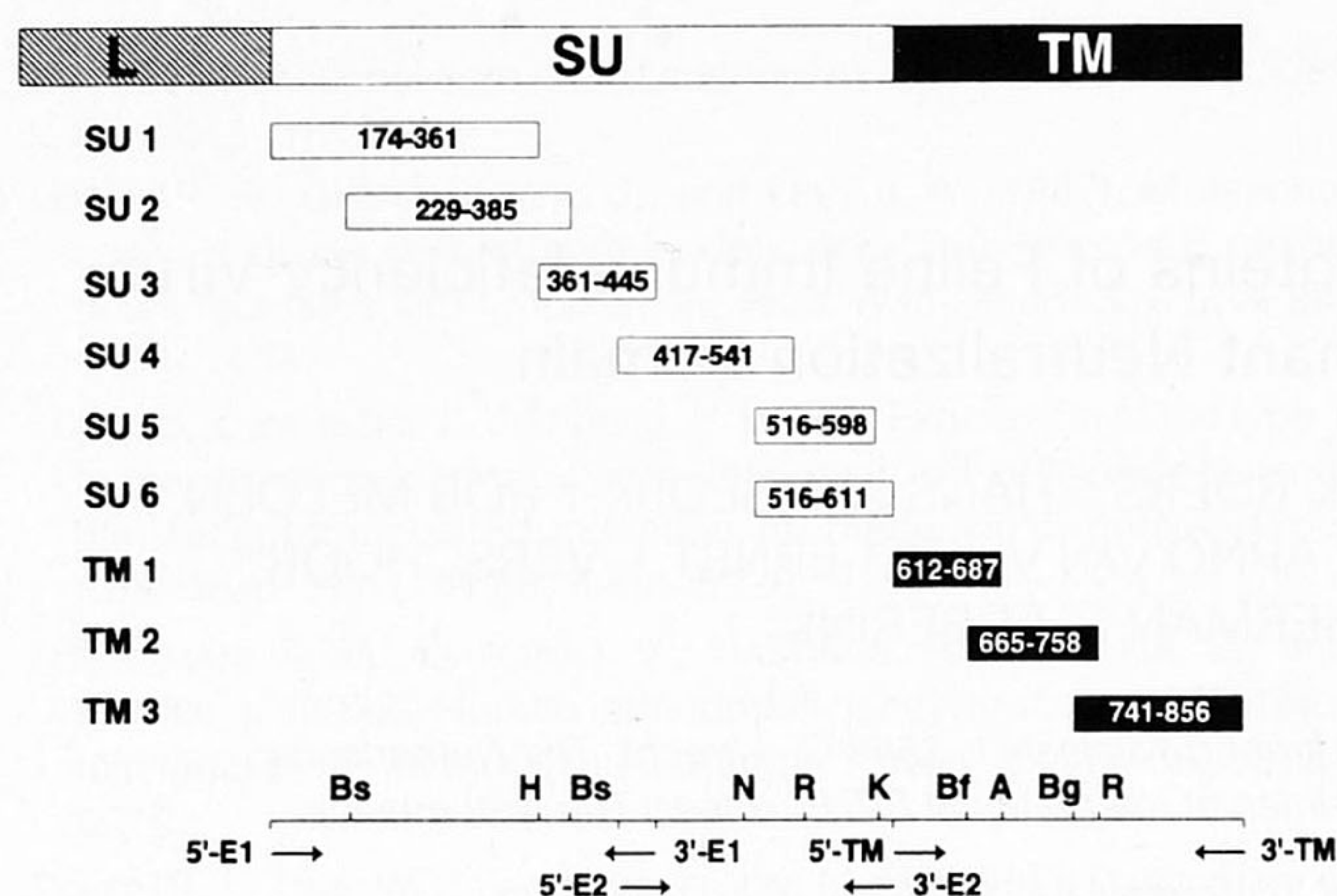


FIG. 1. Map of envelope fragments. Envelope surface fragments were constructed as described (Materials and Methods) using convenient restriction enzyme sites and primers for PCR. Nomenclature of the fragments runs from the NH<sub>2</sub>-terminus of the envelope surface protein (SU1) to the COOH-terminus (SU6) and from the NH<sub>2</sub>-terminus (TM1) of the envelope transmembrane protein to the COOH-terminus (TM3). Numbering indicates the region of envelope covered by the fragments (in amino acids; numbering starts at the first methionine of the env open reading frame). Restriction enzyme sites and primers which were used for the construction of the fragments are indicated (Bs, *Bsa*II; H, *Hind*III; N, *Nsi*I; R, *Rsa*I; K, *Kpn*I; Bf, *Bfr*I; A, *Apo*I; Bg, *Bgl*II).

nal part of the surface env protein starting from the putative cleavage site between leader and SU protein. Similarly, the galk-SU-env fusion encoding the COOH-part of the surface env protein was constructed using PCR with the COOH-end at the cleavage site between the SU and TM envelope protein. PCR was performed in a 100- $\mu$ l reaction mixture containing 50 ng of template (pFIV-UT113-env), 50 mM KCl, 10 mM Tris, pH 8.3, 3 mM MgCl<sub>2</sub>, 10 pmol of each primer, 200  $\mu$ M of each dNTP, and 2.5 U of Taq polymerase (Promega). PCR encompassed 35 cycles of 45 sec at 94°, 1 min at 55°, and 1 min at 72° with an extension of 7 min at 72° at the end of the last cycle. Primers used were 5'-E1: GAT CCATGG CT CAG GTA GTG TGG AG (FIV env nucleotide 520-536; nt 1 is at the A of the first ATG of the FIV-UT113 env open reading frame); 3'-E1: CCT GAATTCTCGAG TTA ACA TCC AAA CTC TAT CC (nt 1335-1319); 5'-E2: CCT GATATCCATGG GA TGT AAT AGC ACA AGA AAT C (nt 1249-1267); 3'-E2: CGC GCTAGC G TTT TCT TCT AGG TTT ATA TTC (nt 1833-1810; nt 1833-1831 were mutated, but still encode an arginine; boldface indicates stop codon; restriction enzyme sites are in italic). The galk-SU-env constructs were as follows: K-SU1 (FIV env nucleotide 520-1083; *Nco*I of primer 5'-E1 to *Hind*III); K-SU2 (nt 685-1155; *Bsa*II fragment); K-SU3 (nt 1083-1335; *Hind*III to primer 3'-E1); K-SU4 (nt 1249-1623; *Nco*I of primer 5'-E2 to *Rsa*I); K-SU5 (nt 1546-1794; *Nsi*I to *Kpn*I); K-SU6 (nt 1546-1833; *Nsi*I to primer 3'-E2).

Plasmids encoding a fusion protein of glutathione S-transferase (GST) and parts of the TM-env protein

were constructed in the pGEX system (Smith and Johnson, 1988) using standard cloning techniques similar to those for the galk expression system. The pGEX plasmid encoding the NH<sub>2</sub>-terminal part of the transmembrane protein as well as that encoding the COOH-terminus of TM were constructed using PCR. PCR primers were 5'-TM: GTACCATGGCAGCTATTCATGTTATG (nt 1834-1851), and 3'-TM: CCCGGA-TCCTCATTCTCCTCTTTTTC (nt 2571-2554). The pGEX-TM-env constructs were as follows: G-TM1 (nt 1834-2060; *Nco*I of 5'-TM to *Apo*I); G-TM2 (nt 1993-2273; *Bfr*I to *Rsa*I); G-TM3 (nt 2221-2571; *Bgl*II to 3'-TM). To allow comparison between the galk and GST systems the SU3 and SU6 fragments were cloned in the GST system as well (G-SU3 and G-SU6).

### Fusion proteins

The expression and purification of galk-SU-env fusion proteins was performed as described (Aldovini *et al.*, 1987; Krone *et al.*, 1988). Briefly, fusion protein expression was induced by adding 60  $\mu$ g/ml nalidixic acid to exponentially growing cultures of transformed AR120 bacteria. Four hours after induction of expression bacteria were lysed by sonication and protein was purified as described (Krone *et al.*, 1988) using preparative SDS-PAGE and electroelution. Fusion proteins of glutathione S-transferase and parts of the env protein were produced by induction of transformed HB101 bacteria with isopropyl  $\beta$ -D-thiogalactoside (0.1 mM). Purification of the GST fusion proteins was performed similar to that of the galk system. Purity of the fusion protein checked on Coomassie-stained SDS-PAGE gels was >95%; protein concentration was estimated by Coomassie blue staining and by the BCA protein assay (Pierce).

To obtain the purified proteins without SDS, which is toxic for cells in the neutralization assays, a somewhat different procedure was used; this alternative procedure is only suitable for fusion proteins with a high expression level. The purified inclusion body fraction, which was normally used for preparative SDS-PAGE, was solubilized in 6 M urea. Urea was removed by stepwise dialysis, and the fusion protein (in 50 mM Tris, pH 8.7/0.5 mM EDTA/0.1 mM DTT/5% glycerol, approximately 80% pure for G-SU3 and G-TM1) could be used in neutralization assays.

### ELISA

Microtiter 96-well plates (Greiner, high bond) were coated overnight at 4° with the SDS-PAGE-purified galk-SU-env or GST-env fusion protein [100 ng per well in phosphate-buffered saline (PBS)]. To check the coating efficacy for the different fusion proteins, a rabbit serum directed against the galk or GST common part of the fusion protein was used. Residual protein



was removed by a wash procedure consisting of three washes with PBS containing 0.05% Tween 20 and two washes with PBS. The plates were blocked with PBS/0.05% Tween 20/5% goat serum at 37° for 1 hr. Blocking was followed by another wash procedure. Sera were diluted in PBS/0.05% Tween 20/5% goat serum (1:100 for cat sera in the galk system and 1:400 in the GST system) and incubated at 37° for 1 hr. After another wash procedure the plates were incubated with a horseradish peroxidase-labeled goat anti-cat serum (Cappel, diluted 1:9000 in PBS/0.05% Tween 20/5% goat serum) at 37° for 1 hr. Horseradish peroxidase activity was detected by incubation with 0.006% H<sub>2</sub>O<sub>2</sub>/100 µg/ml tetramethylbenzidine (Sigma)/100 mM sodium acetate/citrate, pH 5.5. The reaction was stopped by addition of 2 M H<sub>2</sub>SO<sub>4</sub> and was standardized against a series of dilutions of a known positive cat serum. Optical density of the samples was determined at 450 nm in an ELISA reader (Titertek, Multiscan Plus). Cut-off value was calculated as the mean OD of 20 FIV antibody-negative sera plus three times the standard deviation.

### Immunization of rabbits

Rabbits (New Zealand White) were injected subcutaneously with 100 µg of the galk-SU-env or GST-TM-env fusion protein in Freund's complete adjuvant. Every 3 weeks the rabbits received a booster immunization (at least two) with 100 µg of the env fusion protein in Freund's incomplete adjuvant. As shown by immunoblotting and ELISA, hyperimmune sera contained antibodies to the FIV envelope protein as produced in a baculovirus expression system. An exception was the anti-G-TM1 serum which did not react in either system. However, sequencing proved that the GST-TM1 fusion was in frame and able to express the TM1 protein.

### Immunization of cats

Outbred specific pathogen-free cats were injected subcutaneously with 100 µg of the galk-SU3 protein in an alum/oil adjuvant supplemented with *N*-acetyl-D-glucosaminyl-β-(1,4)-*N*-acetylmuramyl-L-alanyl-D-isoglutamine (250 µg/ml). The cats received a booster immunization after 6 and 10 weeks. Hyperimmune sera reacted with the FIV envelope protein as produced in a baculovirus expression system.

### PEPSCAN

The PEPSCAN technique was performed as described (Geysen *et al.*, 1985). Basically, env derived overlapping 12-mer peptides of SU3 (FIV env amino acids 361–372, 362–373, etc., to 433–445) were synthesized on a solid support and each was tested by

ELISA for its capacity to bind antibodies present in sera.

### Neutralization assay

At Day 1, CRFK cells (Crandell *et al.*, 1973) were seeded into a 96-well plate (3500/well) and maintained in DMEM supplemented with 5% fetal calf serum. At Day 2, 50 TCID<sub>50</sub> of CRFK-derived FIV-UT113 were incubated for 1 hr at 37° with serial dilutions of the serum to be assayed. CRFK cells were washed with PBS + DEAE-dextran (50 µg/ml) and incubated overnight with the virus/serum mixture. At Day 3, CRFK cells were washed with PBS and subsequently propagated in DMEM supplemented with 2% fetal calf serum. At Day 8, the culture supernatant was assayed for FIV p24 production. Neutralization was scored positive at >90% inhibition of p24 production.

### Absorption of neutralizing activity in sera

To absorb antibodies, fusion proteins (at various concentrations) purified by the urea solubilization method were incubated for 2 hr with an equal volume of cat serum which was diluted to the reciprocal of the neutralizing titer. The mixture was incubated with virus and processed as usual in the neutralization assay.

### Immunoblotting

Proteins separated by SDS-PAGE were transferred to nitrocellulose sheets (Towbin *et al.*, 1979). Nitrocellulose sheets were incubated with serum as described (De Ronde *et al.*, 1989) and bound antibody was detected with an HRP-labeled anti-cat (Cappel) or anti-rabbit (BioRad) serum.

## RESULTS

### Fusion proteins

The production in *E. coli* of fusion proteins containing parts of the FIV-UT113 envelope proteins was examined by Coomassie blue staining of SDS-PAGE gels and by immunoblotting using sera directed against the galk or GST common part of the fusion proteins. The galk-SU-env fusion proteins showed considerable variations in expression level but were sufficiently abundant to allow their purification. Fragments of the TM protein appeared to be expressed more efficiently as GST than as galk fusion proteins. After electroelution the purity of the fusion proteins was >95% as assessed in Coomassie blue-stained SDS-PAGE gels.



TABLE 1  
ANTIBODY RESPONSE AGAINST ENVELOPE FRAGMENTS IN FIV-INFECTED CATS

Cat	Isolate	Envelope region-specific antibodies								
		SU1	SU2	SU3	SU4	SU5	SU6	TM1	TM2	TM3
14.1	UT-113			++++	+++		++++		++++	
15.1				++++	++++		++++		++++	
16.1				++++	+				++++	
17.1				++++	+		+++		++++	+
18.1				++++	++		++++		++++	
20.1				+++	++		++++		++++	+
21.1				+++	++++				++++	
18.2				++			++		++++	
19.2				+					++++	
Ko	UT-48	++++	++++	+	++++		++++	+	++++	
Bi		++++	++	+++	++++	++	++++		++++	++
340				++++			+		++++	
342				++++			++++		++++	
352				++++	++++		+		++++	++++
356				+++					+++	
308				++++	++++				++++	+
320		++++	+++	++	++++	++++	++++		++++	
322				++++	++				++++	
326				++++	++++		++++		++++	
330				++++	+++		+	+++	++++	
336				+++	++++			+	++++	
831				++++				+	++++	+
833				++++		+			++++	
199				++++			++++		++++	+
201	Petaluma			+			++++		++++	
5		+	+		++++		++++	+	++++	+
6							++++		++++	+
12				++++			+++	+	++++	+
14	clone 19K1			+++			+		++++	+

Note. Sera of cats infected with FIV isolates [the Dutch UT113, UT48, AM-19, and clone 19K1 and the prototype Petaluma (Siebelink *et al.*, 1992; Pedersen *et al.*, 1987)] were screened by ELISA for antibodies against the SU protein fragments SU1 to SU6 and against the TM protein fragments TM1, TM2, and TM3. The strength of the reaction was expressed according to optical density values (no symbol, OD is below cut-off; +, OD is between cut off value and 0.4; ++, OD = 0.4–0.6; +++, OD = 0.6–0.8; +++++, OD > 0.9).

The fusion proteins were used to immunize rabbits and to perform ELISA testing of cat sera.

#### Antibodies against SU-env proteins in cat sera

The galK-SU-env fusion proteins were used to develop an ELISA for the detection of SU-specific antibodies in cat sera; to detect TM-specific antibodies, the GST-TM-env fusion proteins were used. The ELISA based on the GST fusion proteins appeared to be more sensitive when serial serum dilutions were compared in an ELISA with either the galK or GST-SU3 and SU6. To compensate for the difference in sensitivity, cat sera were used at a 1:100 dilution in the galK system and at 1:400 in the GST system. Under these conditions the sensitivity of the assays was not maximal but allowed discrimination between antibody levels to the defined envelope fragments in the different sera. Before infection with FIV, the tested cats did not show FIV antibodies to the SU or TM proteins, indicating the

specificity of the ELISA. All 29 cats seroconverted with antibodies to at least one of the SU and TM proteins (Table 1). Seroconversion started at Week 6 after infection, depending on the isolate and dose of inoculation used. All 29 sera of FIV-infected cats contained antibodies to the TM2 fragment. Most cats (27 of 29) possessed antibodies to SU3, albeit with considerable variation in their levels. The third best recognized protein was SU6 against which antibodies could be detected in 21 of 29 cat sera. SU6 differs from SU5 by a COOH-terminal extension of 13 amino acids. SU5 was recognized by only 3 of 21 cat sera which reacted with SU6, indicating that the 13 COOH-terminal amino acids of the SU protein contribute crucially to this major antigenic site. The envelope fragments TM2, SU3, and SU6 being the most immunodominant ones were also the ones most frequently recognized early after infection. The envelope fragment SU4 was recognized by 17 of 27 cats. The NH<sub>2</sub>-terminal part (SU1 and SU2) of the surface envelope protein was less antigenic and



TABLE 2  
NEUTRALIZATION OF CAT AND RABBIT SERA AGAINST  
ENVELOPE SURFACE FRAGMENTS

Serum	Neutralization titer
Rabbit 2279 $\alpha$ -K-SU1	<10
Rabbit 2193 $\alpha$ -K-SU2	<10
Rabbit 2121 $\alpha$ -K-SU3	320
Rabbit 2195 $\alpha$ -K-SU3	80
Rabbit 2194 $\alpha$ -K-SU4	<10
Rabbit 1448 $\alpha$ -K-SU5	<10
Rabbit 2218 $\alpha$ -K-SU6	<10
Rabbit 2243 $\alpha$ -G-TM1 <sup>a</sup>	<10
Rabbit 2244 $\alpha$ -G-TM2	<10
Rabbit 2245 $\alpha$ -G-TM3	<10
Cat 6 $\alpha$ -K-SU3	80
Cat 8 $\alpha$ -K-SU3	10
Cat 9 $\alpha$ -K-SU3	10-20
Cat 10 control	<10
Pool FIV+ cats	160-320

*Note.* Sera were tested in the neutralization assay. The neutralization titers of preimmune rabbit and cat sera were less than 10. Hyperimmune sera were derived from rabbits which had at least three immunizations; the cats had been immunized three times. The pool of sera of FIV-infected cats was derived from cats infected with FIV-UT48 (cats 320, 322, 326) and had a neutralizing titer which was relatively high among FIV-infected cats tested so far.

<sup>a</sup>  $\alpha$ -G-TM1 did not react with env as expressed in CRFK cells or in a baculovirus system; all other rabbit sera contained FIV envelope protein specific antibodies as measured in an ELISA using a baculovirus-expressed FIV envelope precursor protein.

was recognized by only 4 of 27 cat sera. The cat sera which recognized the NH<sub>2</sub>-terminal part of SU did not or relatively poorly react with the SU3 protein. The TM1 protein was recognized by 6 of 29 sera and the TM3 protein by 11 of 29 sera.

### Neutralization by sera directed to distinct parts of SU

To identify a biological relevant role of antibodies against the distinct envelope protein fragments, polyclonal sera against the SU and TM fragments were raised in rabbits. These polyclonal sera were assayed for neutralizing activity (Table 2). The serum of a rabbit immunized with fusion protein K-SU3 induced neutralizing titers comparable to those in serum from naturally infected cats, whereas sera of rabbits immunized with other parts of the envelope protein did not contain significant neutralizing titers. This indicates that the fusion protein K-SU3 contains one or more epitopes inducing neutralizing antibodies.

To verify that the K-SU3 protein was antigenic in cats as well, it was used to immunize cats. After two immunizations it had induced neutralizing antibodies albeit at titers which were on average lower than in the rabbits which received multiple booster injections using a different adjuvant.

### Absorption by SU3 of neutralizing antibodies in cat sera

To verify that the SU3 fragment constitutes a neutralization domain in infected cats, sera were tested in the neutralization assay after absorption with G-SU3 and as a control with G-TM1 (both purified by the urea solubilization method). The neutralizing activity of 3 of 6 sera could be completely absorbed by SU3 and of two sera partially (Table 3), indicating that the SU3 domain is important in FIV neutralization in infected cats, too.

### PEPSCAN of sera with neutralizing activity

To identify the amino acids crucial to the neutralization domain SU3, sera were assayed using the PEPSCAN technique. The neutralizing rabbit serum, three representative neutralizing cat sera, and a preinfection cat serum were tested by PEPSCAN covering the envelope surface amino acid sequence contained within the K-SU3 fusion protein (Fig. 2). The neutralizing rabbit serum recognized peptides located at the NH<sub>2</sub>-terminal part of K-SU3 which are included in the K-SU2 fusion protein as well. Both the rabbit serum and two of three neutralizing cat sera recognized peptides with the core sequence WRPDFESERV (env amino acids 401-410). In that region of the SU the cat serum 20.1 recognized a wider spectrum of peptides which apparently consisted of multiple core sequences encompassing the SWKQGNRWEWRPDFESERV stretch of

TABLE 3  
ABSORPTION OF NEUTRALIZING ACTIVITY BY ENVELOPE FRAGMENTS

Serum	V.N. titer	Viral replication			
		Protein used for absorption			
		Medium	G-SU3 (1 mg/ml)	G-SU3 (0.1 mg/ml)	G-TM1 (1 mg/ml)
14.1	80	-	-	-	-
20.1	160	-	+	±	-
340	80	-	++	++	-
833	320	-	++	±	-
199	80	-	++	++	-
201	320	-	±	-	-
pre 20.1	<10	++	++	++	++

*Note.* Cat sera were diluted to the reciprocal of the neutralizing titer (except the preinfection serum which was diluted 1:80) and were absorbed with an equal volume of the envelope fragment of the indicated concentration. The serum-envelope fragment mixture was introduced into the neutralization assay. Viral replication was assayed by p24 production (-, no replication/complete neutralization; ±, slightly elevated replication compared to that of neutralizing serum; +, intermediate replication; ++, replication as in the control with preinfection serum of cat 20.1). Absorption of neutralizing antibodies from a neutralizing serum allows viral replication to resume.



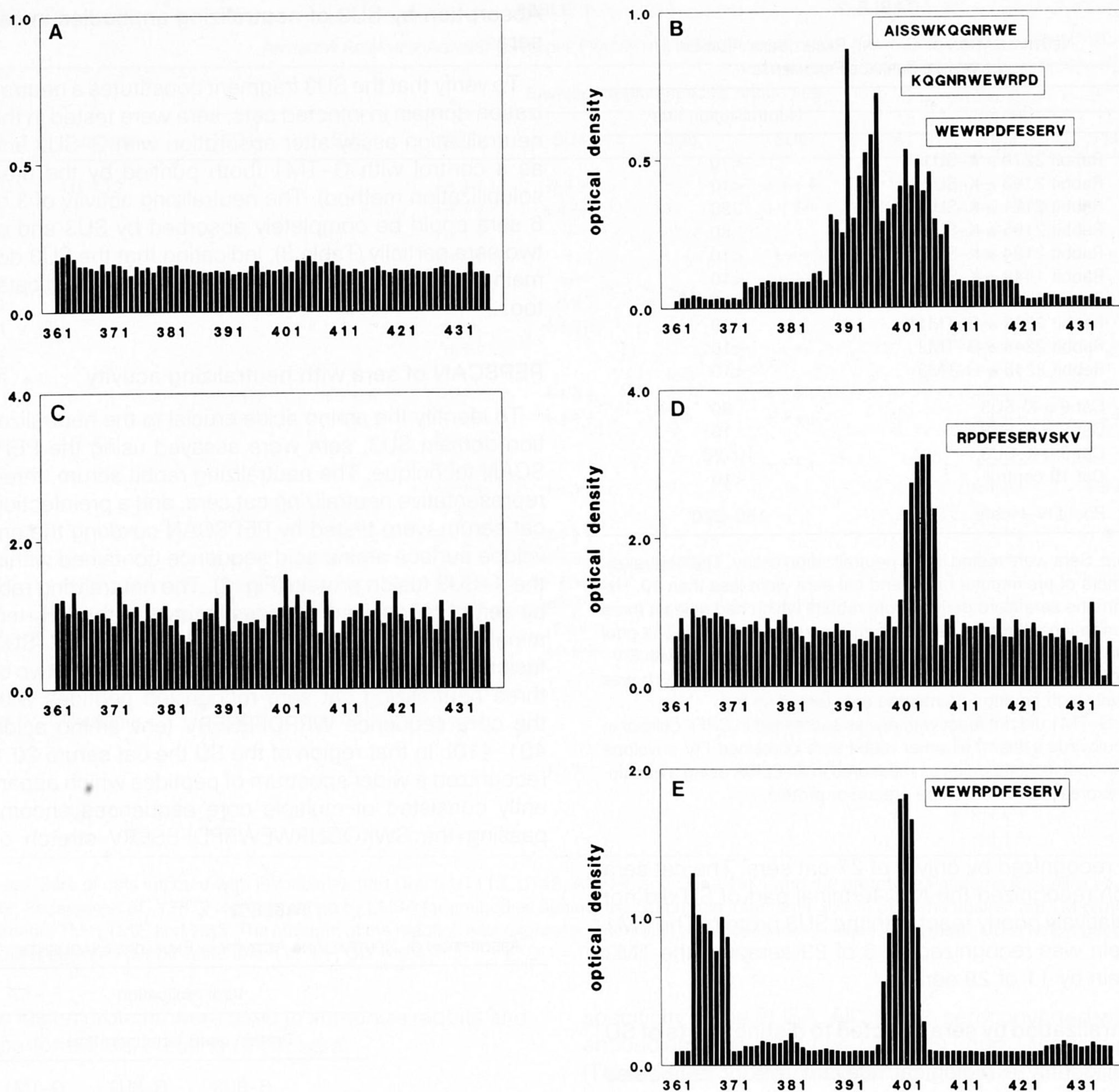


FIG. 2. PEPSCAN analysis of cat and rabbit sera. Overlapping 12-mer peptides of K-SU3 (FIV env amino acids 361–372, 362–373, etc., to 433–445) were synthesized on a solid support and serum antibodies were detected using ELISA. A, a preinfection cat serum; B, serum of an FIV-infected cat (20.1, Table 3); C, id. (cat 199); D, id. (cat 201); E, neutralizing rabbit serum (2121, Table 2).

amino acids (env amino acids 393–411) and including the WRPDFESERV core sequence. The neutralizing cat serum 199 did not react in the PEPSCAN; however, it strongly reacted in the ELISA to SU3 and its neutralizing activity could be absorbed by SU3 (Tables 1 and 3). This indicates that neutralizing antibodies to SU3 can be directed to conformational epitopes which are not detected by PEPSCAN.

### DISCUSSION

We have localized antigenic sites on the envelope SU and TM protein of FIV which elicit antibodies in the

FIV-infected cat. To map the antigenic sites we used overlapping envelope protein fragments that were produced in *E. coli*. An approach that primarily results in the detection of antigenic sites consisting of linear B-cell epitopes. However, like conformational epitopes linear B-cell epitopes can play an important role in eliciting enhancing and neutralizing antibodies as evidenced by, e.g., the V3-loop on the envelope surface protein of HIV-1 (Palker *et al.*, 1988; Goudsmit *et al.*, 1988; Rusche *et al.*, 1988).

The immunodominant site on the envelope proteins of FIV recognized by sera of infected cats is located on



the G-TM2 protein. The location of this immunodominant antigenic site of FIV in the TM2 region of the transmembrane protein has been proposed by Fontenot *et al.* (1992) on the basis of a general model for retrovirus transmembrane proteins (Gallagher *et al.*, 1989) and was supported by serology of FIV-infected cats using a synthetic peptide (Fontenot *et al.*, 1992). The core sequence of the TM2 immunodominant site has been assigned to the QNQFF peptide [amino acids 701–705, (Pancino *et al.*, 1993)]. Its immunodominance makes the TM2 region a good candidate for incorporation in diagnostic assays.

The immunodominant domain of the envelope surface protein is contained within the SU3 region. The SU3 region overlaps the region which has been assigned (Phillips *et al.*, 1990) as the variable region V3 of the envelope (the V1 and V2 region are located in the leader of the envelope precursor). The K-SU3 protein being immunodominant in cats appeared to be the only envelope surface fragment capable of eliciting neutralizing antibodies in rabbits. Neutralizing mouse monoclonal antibodies raised against a  $\beta$ -galactosidase-env fusion protein (Mermer *et al.*, 1992) or the glycosylated FIV envelope (H. F. Egberink *et al.*, unpublished observations) bind either directly to the SU3 protein or to a conformational epitope involving SU3 (H. F. Egberink *et al.*, unpublished observations). Moreover, the K-SU3 protein was antigenic in cats and elicited neutralizing antibodies. The neutralizing activity of three of six sera of FIV-infected cats could be completely absorbed by SU3, while it could be partially absorbed from two sera. This shows that a major neutralization domain of FIV is contained within SU3. Detailed analysis by PEPSCAN indicates that the antibodies in neutralizing rabbit and cat sera are directed to the central region of SU3 (amino acids 390–411). However, one of three cat sera with neutralizing antibodies to SU3 did not react in the PEPSCAN. As the PEPSCAN detects antibodies to linear epitopes, this indicates that conformational as well as linear epitopes within SU3 elicit neutralizing antibodies upon FIV infection. In summary, cat, rabbit, and mouse are able to produce antibodies which neutralize FIV and which are directed against the SU3 region of the envelope protein.

The 13 COOH-terminal amino acids of the envelope surface protein as contained within K-SU6 are recognized by the majority of sera of infected cats. An antigenic site at a similar position has been identified using synthetic peptides (Avrameas *et al.*, 1992). The biological role, if any, of antibodies against the COOH-terminus as well as that of antibodies against the TM2 and the less immunogenic antigenic sites needs to be elucidated.

The identification of the immunodominant site of FIV within the TM protein and the neutralization domain and the COOH terminus as the two major antigenic

sites of the SU is reminiscent of HIV-1. In HIV-1 the immunodominant site is located within the TM at a similar position (Gallagher *et al.*, 1989) and the major linear antigenic sites of the SU are within the V3 principal neutralization domain and at the COOH-terminus (Bolognesi, 1989). The FIV envelope protein, like the HIV-1 envelope protein, contains a principal neutralization domain able to elicit antibodies recognizing linear as well as conformational epitopes. Both neutralizing antibodies and cell-mediated immunity are probably required for protection to lentivirus infection. Therefore, the FIV neutralization domain is a candidate as a component of an FIV vaccine and may serve as a model for the inclusion of the principal neutralization domain of HIV-1 in a human vaccine.

## ACKNOWLEDGMENTS

We are grateful to Christine Debouck for providing the galK expression system, to Kees Siebelink for providing cat sera, to Margreth Valk for help with primer synthesis, to Brion Mermer for a gift of monoclonal antibodies, to Wouter Puyk for performing PEP-SCANS, and to Adrie Versluis, Herma Boere, and colleagues for expert handling of the rabbits. This work was supported by Intervet International and by Grant 90.008 of the Dutch Ministry of Welfare, Public Health, and Culture.

*Note added in proof.* While this work was in progress Lombardi *et al.* (1993), *J. Virol.* **67**, pp. 4742–4749 have also identified a neutralization site within the SU3 region.

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