

Antibodies neutralizing feline leukaemia virus (FeLV) in cats immunized with the transmembrane envelope protein p15E

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Summary

The feline leukaemia virus (FeLV) vaccines that are currently in wide use are generally poor inducers of virus-neutralizing antibodies, although such antibodies appear after recovering from challenge. However, the presence of neutralizing antibodies in cats recovering from natural FeLV infection clearly correlates with resistance to subsequent infection and passive transfer of antibodies can protect other animals. After demonstrating the induction of neutralizing antibodies in rats and goats immunized with the transmembrane envelope protein p15E of FeLV, cats were immunized with the same antigen. High titres of neutralizing antibodies specific for FeLV were induced and epitope mapping revealed a pattern of recognition similar to that seen following immunization of rats and goats. These epitopes are highly related to epitopes recognized after immunization with porcine endogenous retrovirus (PERV) p15E and to epitopes recognized by neutralizing antibodies in patients infected with human immunodeficiency virus type 1. The ability of p15E to induce neutralizing antibodies in cats suggests that it should be included in the next generation of vaccines. In contrast, sera from FeLV-infected animals usually fail to recognize the neutralization-relevant epitopes in p15E. Since homologous epitope sequences are present in feline endogenous retroviruses, it appears that tolerance against these sequences is not induced.

Keywords: neutralizing antibodies; vaccine; retroviruses; transmembrane envelope protein; tolerance

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Introduction

The most effective and efficient method of combating established and newly emerging infectious diseases is the development and use of safe and effective vaccines. This is particularly true for human immunodeficiency virus type 1 (HIV-1) and HIV-2, with 40 million infected people world-wide and 3 million dying from acquired immune deficiency syndrome (AIDS) in 2004 alone. Since the correlate of protection remains elusive, current vaccine efforts concentrate on the induction of both cell-mediated responses and neutralizing antibodies. Since HIV-1, like all retroviruses, inserts proviral DNA into the genome of the infected cell where it may persist for years, induction of neutralizing antibodies able to prevent the initial infection of the cell would be highly effective. In addition, the transmembrane envelope protein (gp41 in

the case of HIV-1) appears to be a better target than the surface envelope protein gp120. Not only is gp41 less variable than gp120, but antibodies binding highly conserved domains of gp41 have been isolated from HIV-1-infected patients that can also neutralize a broad range of HIV strains.^{1,2}

One such antibody, 2F5, binds the epitope ELDKWA and a second, 4E10, binds NWFDTIT. Numerous attempts to induce such antibodies artificially have so far failed and it is unclear whether the broadly neutralizing activity is the result of the complex conformation of the epitope³ or of the extremely long loops in the antibodies.⁴ Recently we induced neutralizing antibodies specific for the transmembrane envelope protein p15E of porcine endogenous retroviruses (PERV)⁵ and feline leukaemia virus (FeLV)⁶ in goats and rats. Two epitopes were mapped, one located in the N-terminal helix and the other in the C-terminal

helix of the transmembrane envelope protein. The epitopes in the C-helix resemble those recognized by 4E10 in the HIV-1 gp41 in terms of both position and sequence. Comparing the p15E and gp41 epitopes and analysing the mode of immunization may be helpful for developing new strategies to induce 2F5/4E10-like antibodies specific for HIV. There is increasing evidence that neutralizing antibodies may prevent HIV infection. Recently it has been shown that regimens involving combinations of human neutralizing monoclonal antibodies specific for conserved, functionally important epitopes in HIV gp120 and gp41, including 2F5 and 4E10, protected monkeys against intravenous and mucosal challenges with simian-human immunodeficiency virus (SHIV) strains (for review see ref. 7).

On the basis of previous experiments in which rats and goats were immunized with FeLV p15E,⁶ we immunized cats to study the immune response in the natural host. In addition, as there is no animal that can be artificially infected with PERV⁸ the cat/FeLV system helps to address the question of whether PERV p15E can induce protective neutralizing antibodies. FeLV is a gammaretrovirus, closely related to PERV, which induces fatal leukaemia, lymphoma and opportunistic infections in infected cats.⁹ At present, two types of FeLV vaccines are available: inactivated whole virus vaccines and subunit vaccines based on the surface envelope protein. There are only limited data concerning the duration of immunity after vaccination with commercially available FeLV vaccines and published studies indicate that 100% protection is achieved by none of them.¹⁰ In this study, high titres of neutralizing antibodies were generated in cats by immunization with p15E and the epitopes mapped were localized in similar domains to those described for rat and goat sera after immunization with p15E. In contrast, sera from cats infected with FeLV recognized different epitopes. It is important to note that cats harbour endogenous retroviruses containing sequences homologous to the epitope sequences. Nevertheless, binding and neutralizing antibodies specific for these domains were induced, suggesting an absence of tolerance. These data indicate that it is possible (and may be necessary to improve vaccines) to induce 2F5/4E10-like neutralizing antibodies against gammaretroviruses and that such epitopes are an effective target for neutralizing antibodies against all retroviruses including HIV.

Materials and methods

Antigen

Affinity-purified recombinant fusion protein p15E of FeLV-A, strain Glasgow, was produced and characterized as described previously.⁶ Briefly, the ectodomain (amino acids 476–583) of p15E of FeLV-A was cloned into the

pCal-n vector (Stratagene, Europe, Amsterdam, the Netherlands), expressed in *Escherichia coli* BL21 DE3 cells. The fusion protein containing p15E fused at its N terminus to a 4000 MW calmodulin-binding protein (CBP) was purified by calmodulin resin affinity chromatography (Stratagene). Protein to be used for immunization was extensively dialysed against phosphate-buffered saline (PBS).

FeLV-infected cats

Sera were obtained from household cats in Germany at the time of first diagnosis of infection using a commercial p27 Gag antigen detection assay (Feline leukemia virus antigen test kit; Symbiotics, Sedona, AZ, USA).

Experimental animals and immunization

Cats, 6–10 months old, were obtained from the University of Düsseldorf and housed in groups of three. They were immunized intramuscularly (i.m.) twice (at weeks 0 and 3) with p15E (Table 1). Montanide[®] ISA 720 (Seppic, Paris, France, lot number 143521) was used as adjuvant mixed with p15E at a ratio of 3 : 7.

SDS-PAGE and Western blotting

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed as described previously¹¹ using 1 µg of the affinity purified recombinant FeLV p15E per lane. Pre- and post-immunization cat sera were titrated in enzyme-linked immunosorbent assay (ELISA) using as antigen the same affinity-purified recombinant p15E used for immunization. Plates (Nunc, Wiesbaden, Germany) were coated for 1 hr at 37° with protein diluted in PBS (100 ng/well). ELISA plates were then washed once with PBS containing 0.1% Tween-20 and blocked for 1 hr at room temperature with PBS containing 0.1% Tween-20 and 5% bovine serum albumin (BSA). Serum samples, diluted in PBS containing 2.5% BSA and 0.1% Tween-20, were added to the ELISA plate at an initial dilution of 1 : 1000 and diluted further in four-fold steps. After incubation for 1 hr at 37°, the ELISA plates were washed three times in PBS containing 0.1% Tween-20 and then a horseradish-peroxidase-conjugated secondary antibody specific for cat immunoglobulin G (IgG; Bethyl, Montgomery, TX, USA), diluted 1 : 3500 in PBS containing 2.5% BSA and 0.1% Tween-20, was added. Incubation for 1 hr at 37° was followed by five washes with PBS containing 0.1% Tween-20. Finally, the ELISA plates were developed by the addition of OPD (α -phenylenediamine dihydrochloride) diluted in PBS (50 µg/well) plus 0.1% H₂O₂ and the reaction was stopped after 10 min by adding 30 µl H₂SO₄ (5 N). Antibody end-point titres are reported as the dilution

Table 1. Characterization of sera from immunized and FeLV- infected cats

Serum	Immunization	FeLV diagnosis	Western blotting p15E	ELISA titre ¹	Neutralization titre ²	Epitope mapping ³			
						E1a	E1b	E2a	E2b
14	p15E	-	+	(6.4 × 10 ⁴) 2.56 × 10 ⁵	(1 : 256) 1 : 1024	++	+	++	-
34	p15E	-	+	(6.4 × 10 ⁴) 2.56 × 10 ⁵	(1 : 256) 1 : 1024	++	+	++	-
44	p15E	-	+	(2.56 × 10 ⁵) 1 × 10 ⁶	(1 : 256) 1 : 256	++	+	++	-
9425	none	+	-	≤ 1 × 10 ³	1 : 16	-	-	-/+	-
6452	none	+	+	4 × 10 ³	1 : 256	-	++	++	-
27470	various vaccines	+	-/+	4 × 10 ³	1 : 256	+	+	-/+	-
54748	none	+	+	4 × 10 ³	0	-	-	+	+
55409	none	+	-/+	4 × 10 ³	1 : 64	-	-	+	+
55284	none	+	-	≤ 1 × 10 ³	1 : 64	-	-	-	-

¹ELISA using recombinant p15E, titres after the booster immunization (titres obtained after first immunization are shown in brackets).

²Inhibition of provirus integration measured by real-time PCR in comparison to preimmune sera, titres after the booster immunization (titres obtained after first immunization are shown in brackets).

³++ strong detection, + weak detection, - no detection.

giving an optical density reading at 492 and 620 nm (OD_{492/620 nm}) above the background of preimmune sera.

Virus neutralization assay

Cell-free supernatant from feline embryonic fibroblast (FEA) cells infected with the FeLV-A Glasgow strain was used as a virus stock in the neutralization assay.⁶ This stock was titrated on uninfected FEA cells and was shown to have a titre of 10⁴⁻⁷⁶ tissue culture infective dose 50%/ml. Neutralization assays were performed as follows. One day prior to the assay, uninfected FEA cells were seeded at 6000 cells per well into 96-well microtitre plates. Seventy-five microlitres of stock virus was incubated with four-fold serial dilutions of serum (previously heat-inactivated at 56° for 45 min) for 45 min at 37° and then transferred to the cells. After 3 days incubation, cells were freeze-thawed three times and lysis buffer containing 20 mg/ml of proteinase K in polymerase chain reaction (PCR) buffer (50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl, pH 8.4) was added. The cells were incubated for 3 hr at 56° followed by 10 min at 95° to inhibit proteinase K activity. Provirus was quantified by real-time PCR as described below.

Real-time PCR

An internal probe FAM-5'-TTAAGCACCTGGGCCCGC-3'-DQ (Eurogentec, Seraing, Belgium) was used together with FeLV-specific primers. The sense primer 5'-TCAAGTATGTTCCCATGAGATACAA-3' and anti-sense primer 5'-GAAGGTGCGAACTCTGGTCAACT-3' were used to amplify and to quantify a 185-base pair (bp) product from the exogenous U3 sequence in the LTR region of the FeLV-A provirus genome. The 25- μ l reaction mixture consisted of 1 × PCR buffer with 1 mM

MgCl₂, 0.5 μ M each of dATP, dCTP, dGTP, dTTP, 5 pmol of each primer, 5 pmol of probe, 1.25 U Ampli-aq Gold polymerase and 2 μ l lysis mixture. The thermal cycling conditions used were 12 min at 95° followed by 50 cycles of 1 min at 95°, 1 min at 59° and 30 seconds at 72° in a Stratagene MX4000 machine.

Epitope mapping

Peptides corresponding to the entire p15E of FeLV-A, Glasgow strain, were synthesized as 15-mer peptides overlapping by 13 amino acids and were covalently linked to a cellulose sheet (Jerini Biotools, Berlin, Germany). Sera diluted 1 : 1000 were incubated with the membrane for 3 hr, washed three times for 15 min with Tris-buffered saline, pH 7.5 containing 0.05% Tween-20 (Sigma-Aldrich, Steinheim, Germany) and incubated for 2 hr with a peroxidase-conjugated secondary antibody diluted 1 : 10 000. Binding was detected using a chemiluminescence detection solution (ECL; Amersham Biosciences Europe, Freiburg, Germany).

Indirect immunofluorescence assay

FeLV-A-producing FEA cells were grown on chamber slides, washed three times with PBS and fixed with 3.5% formaldehyde. Unspecific binding sites were blocked with 5% BSA in PBS for 20 min followed by washing with PBS. Cat sera were applied in 2.5% BSA/PBS at a dilution of 1 : 1000 and incubated at 37° for 1 hr. After five washes with PBS the cells were incubated with fluorescein isothiocyanate (FITC)-labelled goat anti-cat IgG (Bethyl). Finally, the cells were embedded in Prolong[®] anti-fade reagent (Molecular Probes, Invitrogen, Karlsruhe, Germany) and the surface fluorescence was analysed by confocal microscopy (Zeiss, LSM510). Unspecific cell

fluorescence at 543 nm was subtracted from the specific signal at 488 nm.

Results

Induction of binding antibodies specific for p15E in cats

In Western blot analyses, antisera from all three cats immunized with 500 µg p15E (no. 14, no. 34 and no. 44) specifically detected the recombinant p15E protein at a molecular weight of 15 000, while the preimmune sera did not react (Fig. 1a). When sera from FeLV-infected housecats were tested in the same assay, 44 of 75 sera (58.6%) also specifically detected p15E, four of which are shown in Fig. 1(a). FeLV infection had been diagnosed in all animals using a commercial antigen detection assay. These data indicate that immunized and infected animals are able to produce antibodies specific for p15E.

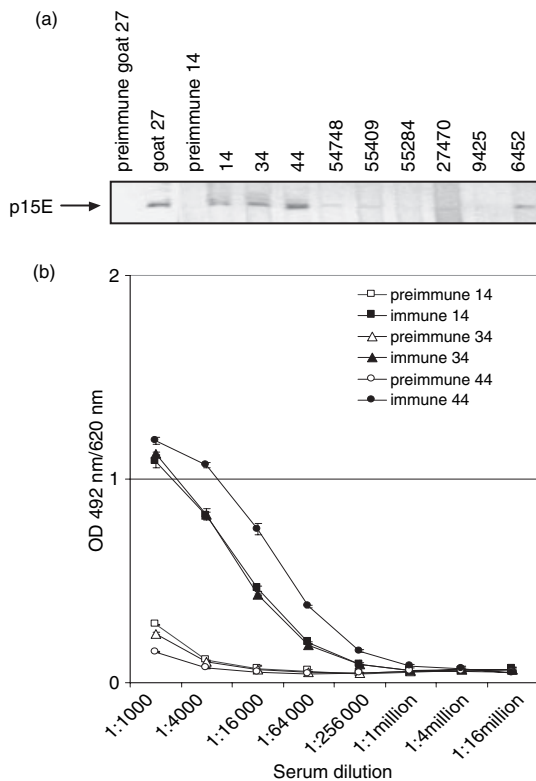


Figure 1. (a) Western blot analysis of cat antisera following immunization with FeLV-A p15E (14, 34 and 44) and of sera from FeLV-infected cats (54748 to 6452). The same recombinant p15E used for immunization was used as antigen. Lane 1 shows the preimmune serum of goat 27, lane 2 shows the corresponding immune serum. Only one preimmune cat serum is shown (no. 14, lane 3). (b) ELISA reactivity of cat sera induced by immunization with 500 µg p15E in comparison with the corresponding preimmune sera. Recombinant p15E was used as antigen.

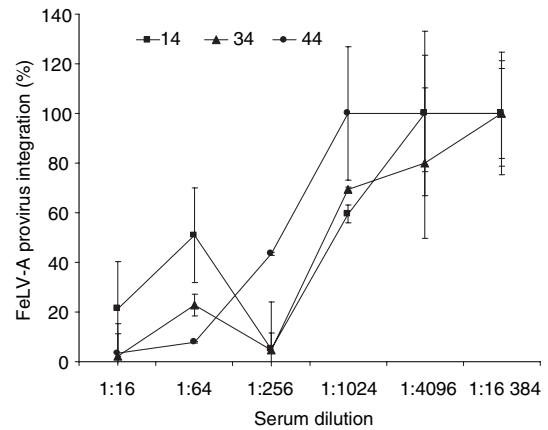


Figure 2. Neutralizing activity of cat antisera after the second immunization with 500 µg p15E. Infection was measured as provirus integration by real-time PCR. Per cent of provirus integration was obtained by comparing antisera with the corresponding preimmune sera.

All three immune sera, but not the preimmune sera, also reacted strongly in ELISA using recombinant p15E as antigen (Fig. 1b). After the boost immunization, the titre of binding antibodies increased markedly (Table 1). Sera from cat 44 showed the highest titres of binding antibodies in this group (2.56×10^5 after the first immunization and 1×10^6 after the boost). The sera from cats 14 and 34 showed titres of 6.4×10^4 that increased to 2.56×10^5 after the boost immunization. In contrast, sera obtained from FeLV-infected housecats had titres between 1×10^3 and 4×10^3 (Table 1).

Induction of neutralizing antibodies specific for p15E in cats

Neutralization of FeLV-A strain Glasgow infection of feline embryonic fibroblast cells was measured using four-fold serial dilutions (from 1 : 16 to 1 : 16384) of the sera. All sera taken after the first immunization had titres of 1 : 256 (Fig. 2) whereas no preimmune sera showed neutralizing activity. Similar to the titres of binding antibodies, the titres of neutralizing antibodies increased after the booster immunization in two animals (cats 14 and 34) up to 1 : 1024 (Table 1) although the titre of neutralizing antibodies in the serum of cat 44 did not increase.

Epitope mapping

To identify the epitopes recognized by the immune sera, epitope mapping was performed using linear 15-mer peptides overlapping by 13 amino acids corresponding to the entire FeLV-A p15E and bound covalently by the C-terminus to a cellulose membrane (Fig. 3a). Four major epitopes were identified (Fig. 3b) using sera from the cats immunized with p15E (no. 14, no. 34 and no. 44). The first epitope, KALLETAQF, is nearly identical to an epitope

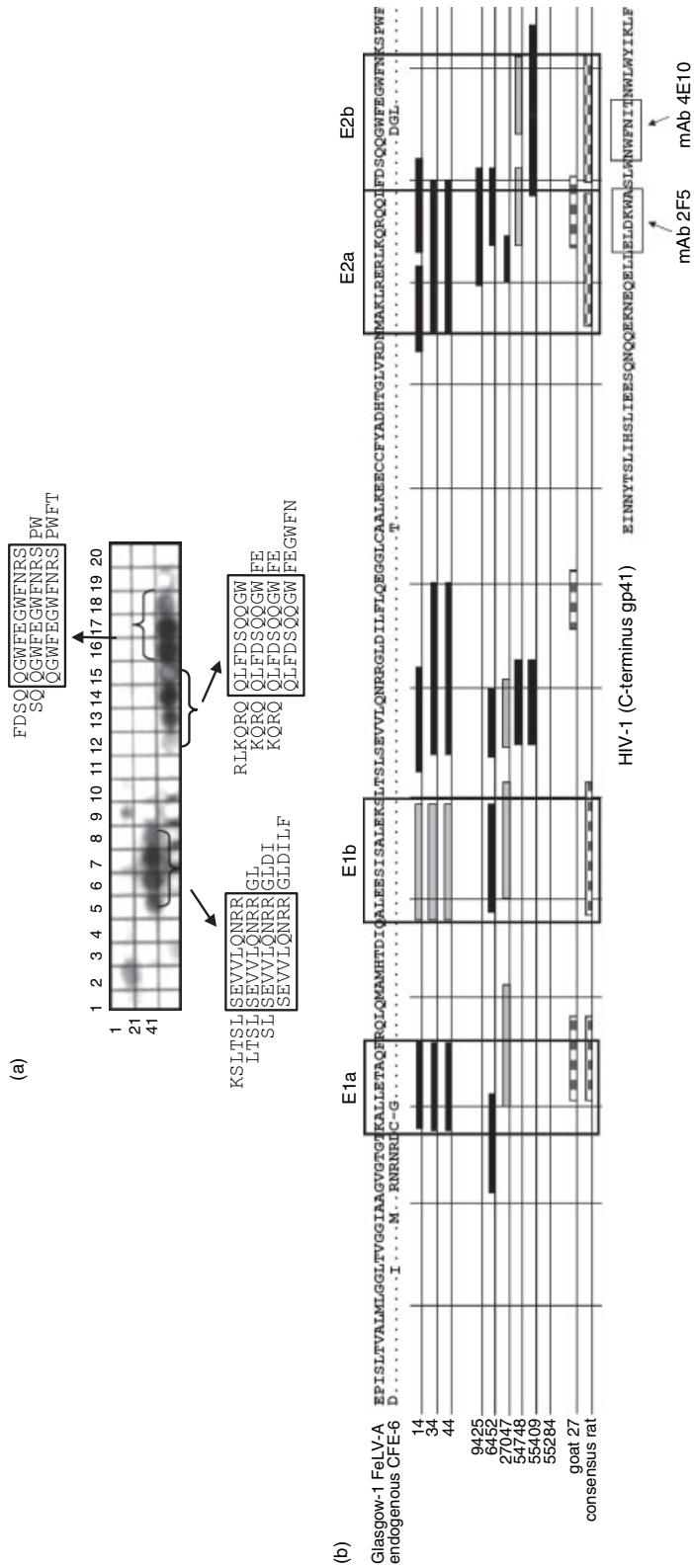


Figure 3. Epitope mapping using sera from immunized and FeLV-infected cats. (a) As an example, binding of serum from FeLV-infected cat no. 55409 to a pepspot membrane carrying overlapping peptides is shown and epitopes are identified. (b) Summary of the epitopes identified recognized by each serum. Sequences corresponding to the recombinant p15E of FeLV-A, strain Glasgow 1, and the corresponding sequence of an endogenous p15E are given at the top. Cats 14, 34 and 44 were immunized with p15E while the others are representative of FeLV-infected cats. Strong epitopes are marked in black, weak epitopes in grey. Common groups of epitopes are framed (E1a, E1b, E2a, E2b). In addition, epitopes recognized by the serum from goat 27, immunized with p15E, and consensus epitopes recognized by eight rats immunized with p15E are shown (hatched).⁶ For comparison, the C-terminal part of the HIV-1 transmembrane envelope protein gp41 and the localization of epitopes recognized by two monoclonal antibodies (mAb) broadly neutralizing HIV-1 (2F5 and 4E10^{1,2}) are shown (framed).

identified by immunizing a goat with FeLV p15E and to a consensus epitope (LETAQFRQL) recognized by sera from eight rats immunized with the same antigen. This epitope group was designated E1a. The second epitope (ALEESI-SALEK, E1b) was also recognized by all eight rat sera but not by the goat serum. The third epitope is located in the immunosuppressive domain, LQNRRGLDILFLQEGGL, which is highly conserved amongst all retroviruses.¹² Synthetic peptides corresponding to this domain inhibit lymphocyte proliferation and modulate cytokine production.¹³ This epitope in the immunosuppressive domain was also recognized by the goat serum, but not by any of the rat sera. The fourth epitope, MAKLRERLKQRQQLF, corresponds to an epitope E2a, recognized both by the goat serum and by seven of eight rat sera. Similar to the goat serum, the cat sera did not recognize an epitope recognized by all rat sera and designated E2b (FDSQQGWFEQWFN). Therefore, the cat sera bound to the main epitopes already described following immunization of rats and goats. These data support the existence of main target epitopes after immunization with p15E and of minor species-specific differences.

Sequences homologous to the epitopes are present in endogenous retroviruses

When the sequence of the FeLV-A p15E used for immunization was compared with that of the endogenous feline retrovirus CFE-6 (NCBI accession no. gi:74706), sequence homologies were identified in the epitope domains (Fig. 3b). Therefore, despite such sequences being present in the genomes of the immunized cats, binding and neutralizing antibodies specific for these domains were induced.

P15E-specific antibodies recognize viral protein at the surface of FeLV-infected cells

To elucidate the possible mechanisms of neutralization, the localization on the cell surface of the epitopes recognized by the p15E-specific sera was analysed by immunofluorescence using non-permeabilized FeLV-infected FEA feline embryonic fibroblast cells. Uninfected cells were not recognized by cat sera from cats no. 14, no. 34 and no. 44 (Fig. 4a). However, all three sera bound to the cell surface (Fig. 4a) whereas the corresponding preimmune sera did not. To increase picture quality, non-specific cell fluorescence at 543 nm was subtracted from the FITC-specific signal at 488 nm. This binding of immune sera to the cell surface indicates that the epitopes identified are accessible to FeLV on the surface of infected cells.

Neutralizing antibodies in the sera of FeLV-infected cats

To compare neutralizing antibody responses in FeLV-infected cats with those of p15E-immunized animals, sera

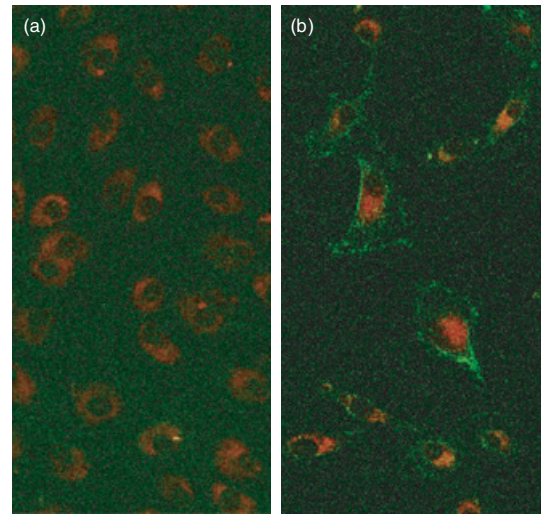


Figure 4. Indirect immunofluorescence, visualized by confocal laser microscopy, using antiserum from cat 44 (immunized with p15E) and FITC-conjugated anti-cat IgG. FITC staining was measured at 488 nm and unspecific fluorescence measured at 543 nm was subtracted. (a) Uninfected FEA cells (b) FeLV-A producing FEA cells.

from infected housecats were analysed. As described above, 44 of 75 sera investigated showed antibodies specific for p15E by Western blot analysis (Fig. 1a) and the titres of p15E-specific antibodies in ELISA ranged from 1×10^3 to 4×10^3 (Table 1). Neutralizing titres of these sera were found to be between 0 and 1 : 256 (Table 1), although it must be kept in mind that in the infected cat neutralizing antibodies might also be directed against other viral proteins such as gp70. Epitope mapping using overlapping peptides spanning the entire p15E (Fig. 3) was carried out. Serum from cat 9425 only recognized the epitope E2a, while serum from cat 6452 recognized an epitope located outside E1a as well as E1b and E2a, and serum from cat 27047 recognized the epitopes E1a and E1b weakly, but E2a more strongly. Cat 27047 had initially been immunized with Leucogen containing the non-glycosylated surface envelope protein p45 but became infected despite this immunization. Sera from cats 54748 and 55409 (Fig. 3a) weakly detected epitopes E2a and E2b but none of these epitopes were recognized by serum from cat 55284, despite this cat being infected and having low titre neutralizing antibodies.

Discussion

This is the first report showing induction of neutralizing antibodies by immunization of cats with FeLV p15E and is in agreement with data obtained by immunization of laboratory rats and goats. The results also agree with data from immunization experiments using p15E of PERV. PERVs represent a clear threat during xenotransplantation of pig cells or organs¹³ because these viruses are present in all pigs and can infect human cells.¹⁴ In addition to

the development of knockout animals lacking replication-competent virus, a protective vaccine might be a useful strategy to prevent PERV transmission. Neutralizing antibodies to PERV have been generated – using p15E – in goats and rats⁵ but there is no animal model available to test the efficacy of such a vaccine.⁸ Numerous attempts to infect minks, rats, mice and non-human primates with PERV were unsuccessful, even when the animals were immunosuppressed and their cells infected *in vitro*^{15–17}. We therefore selected the closely related FeLV as a model to test such immunization strategies *in vivo*. Immunization with p15E of FeLV induced neutralizing antibodies which recognized epitopes similar to those described for sera after immunization with PERV p15E. Although there appeared to be some species-specific epitopes, most epitopes were recognized by rats, goats and cats. However, the titres of neutralizing antibodies achieved in cats was higher than those in rats and goats.

As with PERV,⁵ antisera obtained after immunization with FeLV p15E recognized epitopes located both in the N-terminal helix and the C-terminal helix of p15E. Interestingly broadly neutralizing antibodies isolated from HIV-infected individuals also react with epitopes in similar positions, albeit only in the C-terminal helix (Fig. 3b). One of these antibodies, 4E10, recognizes an epitope with a striking sequence homology given the wide evolutionary gap between HIV and gammaretroviruses (HIV: NWFEDIT, PERV: FEGWFN, FeLV rat: FDSQQGWFEFEGWFN). Interestingly, when immunized with p15E of FeLV, cats and goats did not recognize the E2b epitope, which, in the case of HIV-1, is the 4E10 epitope. Based on our data it seems likely that at least two epitopes have to be recognized to neutralize FeLV or PERV, one in the E1 domain and one in the E2 domain.

Since gp41 or p15E undergo specific conformational changes during the infection process, characterized by binding of the N-terminal helix to the C-terminal helix, it is likely that the E1 epitope and the E2 epitope come into close proximity during this process (Fig. 5). It still remains unclear whether the E1 and E2 domains represent a conformational epitope, e.g. one antibody recognizes both domains, or whether each domain is recognized by its own antibody, one or both of which are responsible for neutralization. Monoclonal antibodies are currently being produced that may help answer this question.

Some commercial vaccines such as Leucogen, which comprise the 45 000-MW unglycosylated surface envelope protein, do not contain p15E and for others containing p15E the role of this protein in the induction of neutralizing antibodies has not been addressed. For the first-generation vaccines that do not fully protect animals from infection, addition of p15E as an additional component may be of great benefit. In preliminary studies, rats immunized with a mixture of p45 and p15E developed higher titres of neutralizing antibodies than animals

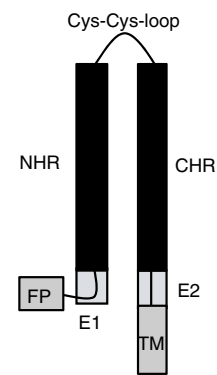


Figure 5. Schematic representation of the transmembrane protein of retroviruses at a defined stage of infection. After introduction of the fusion peptide (FP) the N-helix-region (NHR) and the C-helix region (CHR), which are connected by a cysteine–cysteine loop (Cys-Cys-loop), interact, bringing the epitope regions E1 and E2 into close proximity. TM indicates the transmembrane domain of the protein.

immunized with p45 alone (Langhammer *et al.* submitted for publication). The data presented here demonstrate that p15E is able to induce neutralizing antibodies in cats, the natural host species in which FeLV induces leukaemias and immunodeficiency. Challenge studies in which p15E-immunized cats are challenged with infectious FeLV-A Glasgow are underway to assess the protective effect of vaccination *in vivo*.

Interestingly, cats infected with FeLV also develop antibodies against p15E, although the reactions by immunoblot are weak and ELISA titres are low. Epitope mapping revealed a variety of epitopes recognized by sera from FeLV-infected animals, including epitopes detected by sera from p15E-immunized cats, albeit comparatively weakly. This suggests that natural FeLV infection results in a weak induction of antibodies specific for the viral transmembrane protein p15E and a low induction of neutralizing antibodies.

Comparing the sequence of the infectious FeLV-A, able to induce leukaemia and immunodeficiency in infected cats, with that of an endogenous provirus, revealed a strong homology in the epitopes E1a, E1b, E2a and E2b. Only the E2b sequence (DGL instead of GWF) and regions outside these did the epitopes show differences (Fig. 3b). The induction of binding and neutralizing antibodies specific for sequences present as endogenous retroviruses in the genome of all cats indicates a lack of tolerance and suggests that expression of the endogenous viral genes during ontogeny (when discrimination between self and non-self is made) does not occur. Similar observations have been made with human endogenous retroviruses (HERVs), especially HERV-K, which is expressed in human teratocarcinomas¹⁸ and melanomas.^{19,20} Antibodies against HERV-K were found in 45% (45 of 100) of testicular tumour

patients, 26% (31 of 120) of lymphoma patients and 38% (three of eight) of multiparous pregnant women.¹⁸ Furthermore, antibodies against the transmembrane envelope protein were found in 22% (13 of 60) of melanoma patients.²⁰ Although antibody titres are elevated compared with normal blood donors (3%, one of 30), they hardly ever reach the titres seen after infection with exogenous retroviruses such as HIV. Nevertheless, it is intriguing that antibodies are produced at all, because HERV proteins (like the proteins of endogenous retroviruses in cats) can be regarded as self-antigens that should induce tolerance. However, the presence of antibodies suggests that tolerance is not induced and that induction of antibodies specific for endogenous retroviral proteins is possible both by immunization and by infection with an exogenous but highly related leukaemia virus. Absence of tolerance to retroviral proteins is certainly characteristic for all species carrying endogenous retroviruses.

The mechanism of action of the broadly neutralizing, HIV-1 gp41-specific human monoclonal antibody 2F5 is almost as poorly understood as the mechanism of action of the p15E antibodies neutralizing PERV or FeLV. It is known that 2F5 binds to the virion before attachment of the virus to the cell²¹ and that 2F5 does not influence the interaction between the helices²² but rather inhibits later stages of infection.²³ However, all attempts to induce neutralizing antibodies using recombinant proteins containing the E2 epitope of HIV-1 gp41 (ELDKWA), have so far failed.³ However, recently we were able to induce 2F5/4E10-like antibodies using hybrid proteins containing p15E of PERV or FeLV as backbone as well as sequences corresponding to the 2F5/4E10 epitopes (WO 2005/021574, Fiebig *et al.*, in preparation, Langhammer *et al.*, in preparation). Here, immunofluorescence experiments demonstrated that the FeLV p15-specific antibodies induced in rats⁶ and cats (Fig. 4), like 2F5, are able to bind to the cell surface of infected cells, suggesting the availability of the epitopes on the virion itself despite the fact that most of the transmembrane envelope protein is occluded by the surface envelope proteins gp70 or gp120, respectively.

These data show a greater similarity than previously suspected between gammaretroviruses, such as PERV and FeLV, and lentiviruses, such as HIV-1, with regard to the localization of target epitopes for broadly neutralizing antibodies in the transmembrane envelope proteins. This similarity may be based on a common requirement for conformational changes during retroviral infection that can be inhibited by antibodies and hence prevent later stages of virus internalization.

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