gag- And env-specific serum antibodies in cats after natural and experimental infection with feline immunodeficiency virus

G.F. Rimmelzwaan[a], K.H.J. Siebelink[a], H. Broos[a], G.A. Drost[a], K. Weijer[b,c], R. van Herwijnen[a] and A.D.M.E. Osterhaus[d,*]

[a]*Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, Netherlands*
[b]*Department of Pathology, National Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, Netherlands*
[c]*European Veterinary Laboratory, P.O.Box 198, 3440AD Woerden, Netherlands*
[d]*Department of Virology, Erasmus University, P.O. Box 1738, 3000DR Rotterdam, Netherlands*

Abstract

In order to monitor the antibody response to feline immunodeficiency virus (FIV) in cats, following experimental and natural infection, enzyme-linked immunosorbent assays (ELISAs) were developed using recombinant *env* and *gag* proteins and p24-specific monoclonal antibodies. It was shown that in experimentally infected cats an *env* protein-specific antibody response was directly followed by a *gag* protein-specific response. Furthermore, an ELISA for the detection of *env* protein-specific serum antibodies proved more sensitive in identifying experimentally and naturally infected cats than ELISAs demonstrating *gag* protein-specific antibodies. It was concluded that, like in HIV infection of humans, the detection of *env* protein-specific serum antibodies in addition to *gag* protein-specific antibodies is not only an important tool in the diagnosis of the infection but also in studies concerning the pathogenesis of the disease.

Introduction

Feline immunodeficiency virus (FIV) is a T-lymphotropic lentivirus that was first isolated from domestic cats suffering from clinical signs indicative of an immunodeficiency syndrome (Pedersen et al., 1987). The disease in cats is characterized by lymphadenopathy and severe impairment of immune function (Ackley et al., 1990; Siebelink et al., 1990a;

Corresponding author.

Torten et al., 1991) accompanied by a decrease of the number of CD4 T lymphocytes (Hoffmann-Fezet et al., 1992). FIV has been shown to infect cat macrophages (Brunner and Pedersen, 1989), astrocytes (Dow et al., 1990) and the CD4$^+$ subset of T lymphocytes: a cell tropism similar to that of human immunodeficiency virus (HIV). Thus in many respects FIV infection in cats resembles HIV infection in man and therefore FIV infection should be considered a useful animal model for AIDS in man. The model has been shown suitable to study the potential of certain antiviral compounds (Egberink et al., 1990; Remington et al., 1991), approaches for experimental vaccination (Yamamoto et al., 1991) and the pathogenesis of lentivirus induced immunodeficiency (Yamamoto et al., 1988). For these studies the development of assays to monitor the FIV-specific immune response after FIV infection and immunization with candidate vaccines is of critical importance. Several serological assays which demonstrate antibodies specific for the structural proteins of FIV have been described. These include enzyme-linked immunosorbant assays (ELISAs) based on whole virus preparations and immunofluorescence assays (IFA), both of which do not discriminate between antibodies directed against the individual FIV proteins (O'Connor et al., 1989; Siebelink et al., 1990b). Also Western blot and radio immunoprecipitation assays have been described (Lutz et al., 1988; Hosie and Jarrett, 1990; Egberink et al., 1992), which are, however, too laborious for routine serological procedures. Recently, virus neutralization assays have been developed, which demonstrate antibodies that may directly be involved in protection from infection or development of disease (Tozzini et al., 1992).

With the advent of hybridoma, recombinant DNA and peptide synthesis technologies it has become possible to develop assay systems which can demonstrate antibodies directed to individual proteins or even individual epitopes. In the present paper we describe the comparison of four newly developed ELISAs based on the use of FIV-*env* and p24 recombinant proteins and p24-specific monoclonal antibodies to monitor FIV-specific antibody development in the sera of naturally and experimentally infected cats.

Materials and methods

Virus preparations

An FIV isolate (Adam 4) from a Dutch cat seronegative to FeLV and FeSFV, was obtained by cocultivating peripheral blood mononuclear cells (PBMC) with Concanavalin-A and Interleukin-2 stimulated PBMC from a specified pathogen-free (SPF) cat as previously described (Siebelink et al., 1992). The FIV Petaluma strain was kindly provided by Dr. N.C. Pedersen (Davis, CA, USA). Crandell feline kidney (CrFK) cells (Crandell et al., 1973) were infected with FIV by cocultivation of CrFK cells with the infected PBMC as described (Siebelink et al., 1990b) and cultured in Dulbecco's minimal essential medium containing 100 IU/ml penicillin, 100 μg/ml streptomycin, 0.002 M glutamin and 5% fetal calf serum (FCS). Culture supernatants of the infected (and non-infected) CrFK cells were collected seven to ten days after passage of the cells in fresh medium. The culture supernatants were used as antigens in the CTB-ELISA (see below) after clarification by low speed centrifugation (10 min, 2000 rpm).

For immunization purposes FIV was purified from culture supernatant by density gradient centrifugation. The virus was first pelleted by centrifugation for one hour at 100000 g at

4°C. The pellet was resuspended in phosphate buffered saline (PBS) and then layered over a discontinuous metrizamide (Nyegaard and Co, Oslo, Norway) gradient. After centrifugation for one hour at 100000 g at 4°C, virus containing fractions identified by a previously described ELISA (Siebelink et al., 1990b) were collected.

Concentration and partial purification of FIV antigen from culture supernatant was also performed by precipitation in polymer based aqueous two phase systems as described for other retroviruses (Hammar et al., 1989a; Hammar et al., 1989b; Hammar and Gilljam, 1990). Polyethylene glycol 6000 (Merck, Schuckardt, Germany) and Dextran T 500 (Pharmacia, Uppsala, Sweden) were added to the culture supernatants at final concentrations of 7% and 0.2% respectively. The mixture was allowed to equilibrate by gently rocking for 16 hours at 4°C. Two phases were allowed to settle for another 16 hours at 4°C. Bottom and interphase (containing FIV) and top phase were collected separately. In this way a concentration of FIV of about 100-fold was achieved. This antigen was used for western blot assays (see below).

Generation and selection of monoclonal antibodies (mAbs)

A panel of hybridoma's producing mAbs in ELISA reactive with FIV, was generated essentially as previously described for the generation of mAbs against poliovirus (Osterhaus et al., 1981). Briefly, spleen cells from BALB/c mice, which were subcutaneously immunized with 5 μg gradient purified FIV in Freund's complete adjuvant on day 0 and in Freund's incomplete adjuvant on day 14, were fused with a mouse myeloma cell line after intraperitoneal booster injections on days 28, 29 and 30 with 5 μg of pelleted FIV. Hybridomas producing FIV-specific mAbs were selected by screening culture media in an ELISA. For this ELISA microtiter plates (Costar, High binding RIA/EIA plates) were treated by incubating 50 μl volumes of a PBS solution containing 0.125 mg poly-L-lysine and 10 mg 1-ethyl-3(3-dimethylaminopropyl)-carbodiimidehydrochloride (EDC) (Sigma, St. Louis, USA) per ml, for one hour at room temperature. After each incubation the plates were washed with demineralized water containing 0.05% Tween-80 (Merck, Schuckardt, Germany) (DWT). Fifty μl volumes containing 300 ng of pelleted FIV or control antigen were coated in 0.1 M carbonate buffer, pH 9.6 for 16 hours at 20°C. The plates were blocked with 50 μl volumes of PBS containing 0.05% Tween-80 and 1% bovine serum albumin (BSA) (PBS-TB) for one hour at 37°C. Fifty μl of undiluted culture supernatants were transfered to the wells and incubated for one hour at 37°C. After washing, 50 μl volumes of a horse radish peroxidase (HRP) conjugated goat anti-mouse IgG (Fc) antibody preparation (Cappel, Cooper Biomedical, Malvern, USA) were added to the wells and incubated for one hour. The plates were washed and 100 μl volumes of substrate solution (0.1 mg/ml tetramethyl benzidin (TMB) and 0.003% H_2O_2 in 0.1 M NaAc buffer, pH 5.5) were added to each well. After incubation for 10 min at 20°C 100 μl volumes of 2 M H_2SO_4 were added to stop the color reaction. The absorbance at 450 nm (A450) was read in a Titertek Multiscan (Titertek, Flow Laboratories). Hybridomas were selected and single cell cloned twice by micromanipulation. Mouse ascitic fluids were produced in BALB/c mice. Immunoglobulins (Ig) were isolated from mouse ascitic fluids by precipitation with ammonium sulphate and affinity chromatography using protein A sepharose (Pharmacia Fine

Chemicals, Uppsala, Sweden) (Seppälä et al., 1981). Ig classes and isotypes were determined in an indirect ELISA with specific anti-Ig conjugates (Zymed Laboratories, CA, USA) and are indicated in brackets.

Monoclonal antibodies were conjugated to biotin by incubating the antibody solution, which was dialyzed against 0.1 M NaCO₃, pH 8.3 (1 mg protein per ml) with N-hydroxysuccinimido biotin (Sigma, St. Louis, USA) solubilized in dimethyl sulphoxide (1 mg/ml) at a 1:8 (w/w) ratio (biotin:protein) for four hours at room temperature. The conjugates were dialyzed against PBS and stored at −20°C.

SDS-PAGE and western blot analysis

FIV (Adam 4) preparations were denatured in the presence of sodium dodecylsulphate and 2-mercaptoethanol and the individual viral proteins were separated on polyacrylamide gel (12%) by using the discontinuous buffer system (Laemli, 1970). Polypeptides were then electrically transfered to nitrocellulose sheets (Towbin et al., 1979). For immunostaining the nitrocellulose was blocked with PBS-TB for one hour at 20°C and after each incubation washed three times for 10 min.

After incubation with monoclonal antibodies or cat sera for one hour at 20°C, the nitrocellulose was incubated with horse radish peroxidase (HRP) conjugated goat anti-mouse IgG (Fc) or goat anti-cat IgG (Cappel, Cooper Biomedical, Malvern, USA) respectively. Subsequently the nitrocellulose strips were developed with 24 mg tetramethyl benzidin and 80 mg dioctyl sodium sulfosuccinate diluted in 10 ml ethanol supplemented with 30 ml 0.1 M citrate/phosphate buffer pH 5.5 and 25 μl 30% H_2O_2.

Recombinant FIV proteins

Recombinant FIV *env* protein, derived by PCR amplification of DNA from bone marrow cells of a naturally FIV-infected cat (cat Adam 19), was obtained by expression through recombinant vaccinia virus (rVV) vGR657 as described (Rimmelzwaan et al., 1993). In brief, HeLa spinner cells or BHK spinner cells were infected at a m.o.i. of five with rVV vGR657. After 48 hours, the cells were washed with PBS and lysed in PBS containing 0.5% NP-40 and 0.5% SDS. After centrifugation for 20 min at 10000 × g, the supernatant was used as FIV-*env* antigen in the respective assays.

Recombinant FIV p24 and p17 protein were obtained by expression in *E. coli* as fusion proteins using the pGEX-2T expression plasmid as previously described (Reid et al., 1991).

Cats and serum samples

Six female SPF cats of about 12 weeks old from a breeding colony (Harlan CPB, Zeist, The Netherlands), which is regularly screened for the absence of viral pathogens, were infected via the intravenous route with about 100 TCID₅₀ of one of three different FIV strains. Two (#5 and #6) with the Petaluma strain (kindly provided by Dr. N.C. Pedersen, Davis, CA, USA), two (#11 and #12) with a Dutch isolate (Adam 19) and two (#13 and #14) with the progeny virus of the infectious molecular clone 19K1 that was obtained directly from bone marrow DNA of a naturally infected cat, from which also FIV isolate Adam 19 was obtained (Siebelink et al., 1992). Serum samples were collected at regular intervals after infection, while the animals were kept in strict isolation.

Serum samples were also collected from 11 privately owned Dutch cats which were suspected of FIV infection on clinical grounds and positive serology in a commercial test

(Petcheck, IDEXX Corporation) and from 26 privately owned Dutch cats without clinical signs.

ELISAs for the detection of FIV-specific antibodies

For the detection of FIV-specific antibodies, four indirect ELISAs were used. These ELISAs differed only in the antigen preparations used for the coating of the ELISA plates (Costar RIA/EIA, High binding). Optimal concentrations of the reagents used were determined by checkerboard titration. The coating procedures of the four ELISAs were as follows:

1. *Total virus ELISA (TV-ELISA)*. For the TV-ELISA, FIV antigen for coating was concentrated by ultracentrifugation from supernatants of infected CrFK cell cultures. After solubilization with 1% w/v Triton X-100 (Merck, Schuckardt, Germany), the antigen was diluted in 0.1 M NaAc buffer, pH 5.5, 50 μl volumes were added per well and the plates were incubated at room temperature for 16 hours. The wells were then emptied and fixed with 4% (w/v) paraformaldehyde for 10 min at room temperature. After washing the plates were blocked with PBS containing 0.05% Tween-80 and 10% FCS.

2. *ELISA based on FIV-specific mAbs (MTV-ELISA)*. For the MTV-ELISA microtiter plates were coated with purified mAb 8–21 (see below) in 0.1 M carbonate buffer, pH 9.6. The plates were washed with DWT containing 0.05% Tween-80 after each incubation. After blocking with PBS-TB for one hour at 37°C, 50 μl volumes of culture supernatants of FIV infected (or non-infected) CrFK cells, to which 1% Triton X-100 and 5% NaCl was added, were incubated for one hour at 37°C.

3. *ELISA based on recombinant FIV-env protein (rec.env-ELISA)*. For the rec.*env*-ELISA cell lysates of rVV vGR657 (or wild type vaccinia virus) infected RK13, BHK or HeLa cells were coated in 0.1 M NaAc buffer, pH 5.5 in 50 μl volumes for 16 hours at room temperature. The wells were then emptied and fixed with 4% (w/v) paraformaldehyde for 10 min at room temperature. After washing the plates were blocked with PBS containing 0.05% Tween-80 and 10% FCS.

4. *ELISA based on recombinant FIV p24/p17 proteins (rec.gag-ELISA)*. The rec.*gag*-ELISA, based on recombinant *gag* proteins, was purchased from the European Veterinary Laboratory B.V., Woerden, The Netherlands (FIV-p24/p17 antibody test kit, cat no. F1002-AB01) and performed essentially as described previously (Reid et al., 1991).

After coating and blocking of the microtiter plates, 50 μl of twofold serial dilutions of serum samples in PBS-TB supplemented with 5% NaCl were added at a starting dilution of 1:40 or 1:20. Then the plates were incubated for one hour at 37°C. Subsequently 50 μl volumes of a biotin conjugated mouse mAb directed to cat IgG (Sigma, St. Louis, USA) or an HRP conjugated goat anti-cat IgG antibody preparation (Cappel, Cooper Biomedicals, Malvern, USA) were added and the plates were incubated for one hour at 37°C. HRP bound streptavidin (Amersham, Amersham, UK) was allowed to bind to biotin for 30 min at 37°C. After each of these incubations the plates were washed with DWT and subsequently developed with TMB as described above. OD_{450} values were determined in a Titertek multiscan. Titres were given as the reciprocals of the dilutions still giving more than three times background values obtained with control antigen coated plates.

Complex trapping blocking ELISA (CTB-ELISA) for the detection of FIV p24-specific antibodies

For the CTB-ELISA microtiter plates were coated with FIV p24-specific mAb 8–21 and blocked with PBS-TB as described for the MTV-ELISA. The plates were washed twice after each incubation with DWT. Twenty-five μl volumes of twofold serial dilutions of cat serum samples, starting at a dilution of 1:5 were added simultaneously with 25 μl of a dilution of culture supernatant of FIV infected CrFK cells giving 75% of maximum binding. All dilutions were prepared in PBS-TB supplemented with 1% Triton X-100 and 5% NaCl. After incubation for two hours at 37°C, remaining binding of FIV antigen was detected using biotin conjugated mAb 2–13 (see below). The combination of mAb 8–21 as capture antibody and mAb 2–13 as conjugated antibody was chosen as it proved to be the most sensitive among those tested with the mAbs generated in these studies. After incubation with biotin conjugated mAb 2–13 for one hour at 37°C, HRP bound streptavidin was allowed to bind to biotin for 30 min at 37°C. The plates were developed as described above. Test samples were considered positive if a reduction of 50% or more of the signal was observed.

Results

Selection and characterization of FIV-specific mAbs

Five hybridomas, producing antibodies reactive with FIV antigen as determined in the hybridoma screening ELISA, were obtained from two fusion events. These mAbs were designated 2–11 (IgG1,k), 2–13 (IgG2b,k), 3–20 (IgG2a,k), 4–22 (IgG1,k) and 8–21
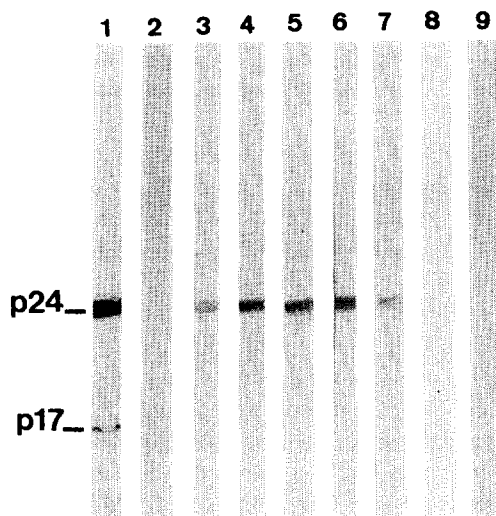

Fig. 1. Western blot analysis of FIV-specific mAbs. Viral proteins of two phase system purified FIV (lanes 1–7) or control antigen (lanes 8 and 9) were separated by SDS-PAGE and electrically transferred to nitrocellulose. For immunostaining individual lanes were incubated with serum of a FIV seropositive cat (diluted 1:100, lanes 1 and 8), serum of a seronegative cat (diluted 1:100, lane 2), culture supernatants of mAbs 2–11 (lane 3), 2–13 (lane 4 and 9), 3–20 (lane 5), 4–22 (lane 6) and 8–21 (lane 7).
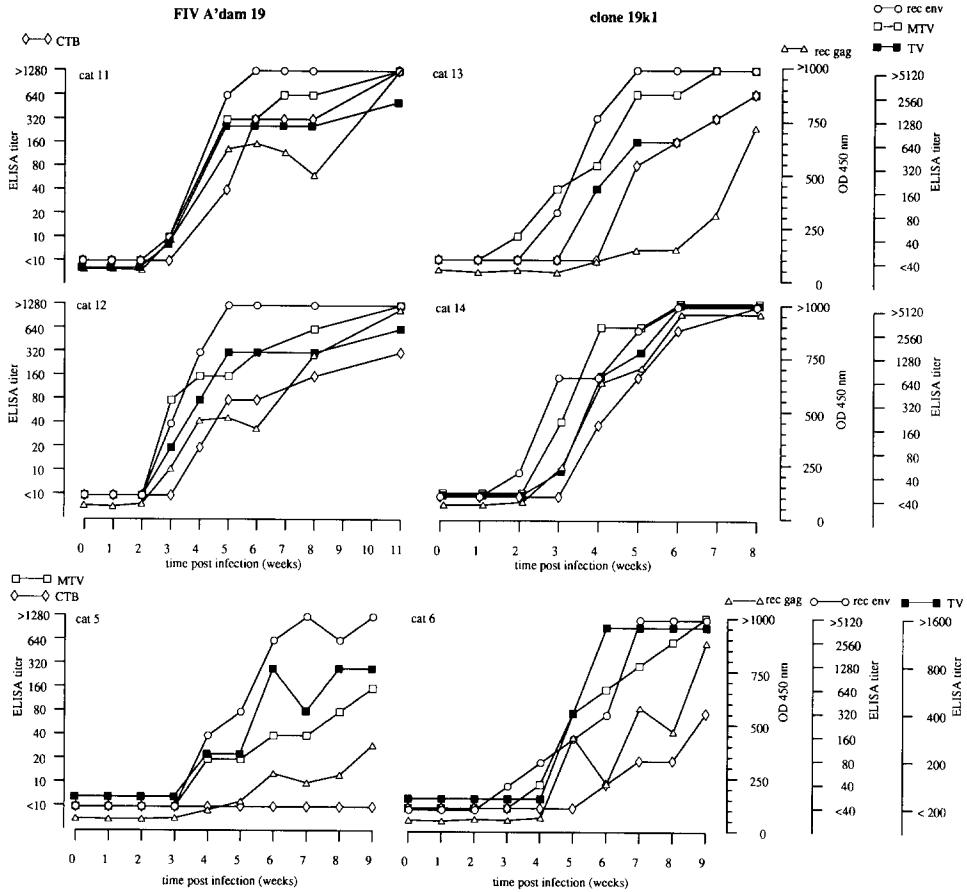
Fig. 2. Development of FIV-specific antibodies in experimentally infected cats. Antibody responses were monitored in the TV-ELISA (-■-), MTV-ELISA (-□-), rec.*env*-ELISA (-O-), rec.*gag*-ELISA (-△-) and CTB-ELISA (-◇-) of: cats infected with the Dutch isolate Adam 19 of FIV (cats #11 and #12), cats infected with the progeny of infectious molecular clone 19K1 (cats #13 and #14) and cats infected with the Petaluma strain of FIV (cats #5 and #6).

(IgG1,k). When tested for their reactivities in Western blot analysis, these five mAbs all reacted with the denatured p24 core protein of FIV (Fig. 1). No reactivity was found with control antigen concentrated and purified in the same way. The specificity of these five mAbs for the FIV *gag* protein was confirmed by demonstrating their reactivities in the rec.*gag*-ELISA ($OD_{450} > 800$ per 50 ng Ig) in which their respective isotype control mAbs did not react (data not shown).

FIV-specific antibody responses in experimentally infected SPF cats

The development of FIV-specific serum antibodies in experimentally infected cats was monitored in the respective ELISAs (Fig. 2). In two SPF cats (#11 and #12) infected intravenously with the Dutch FIV isolate Adam 19 and two other SPF cats (#13 and #14)
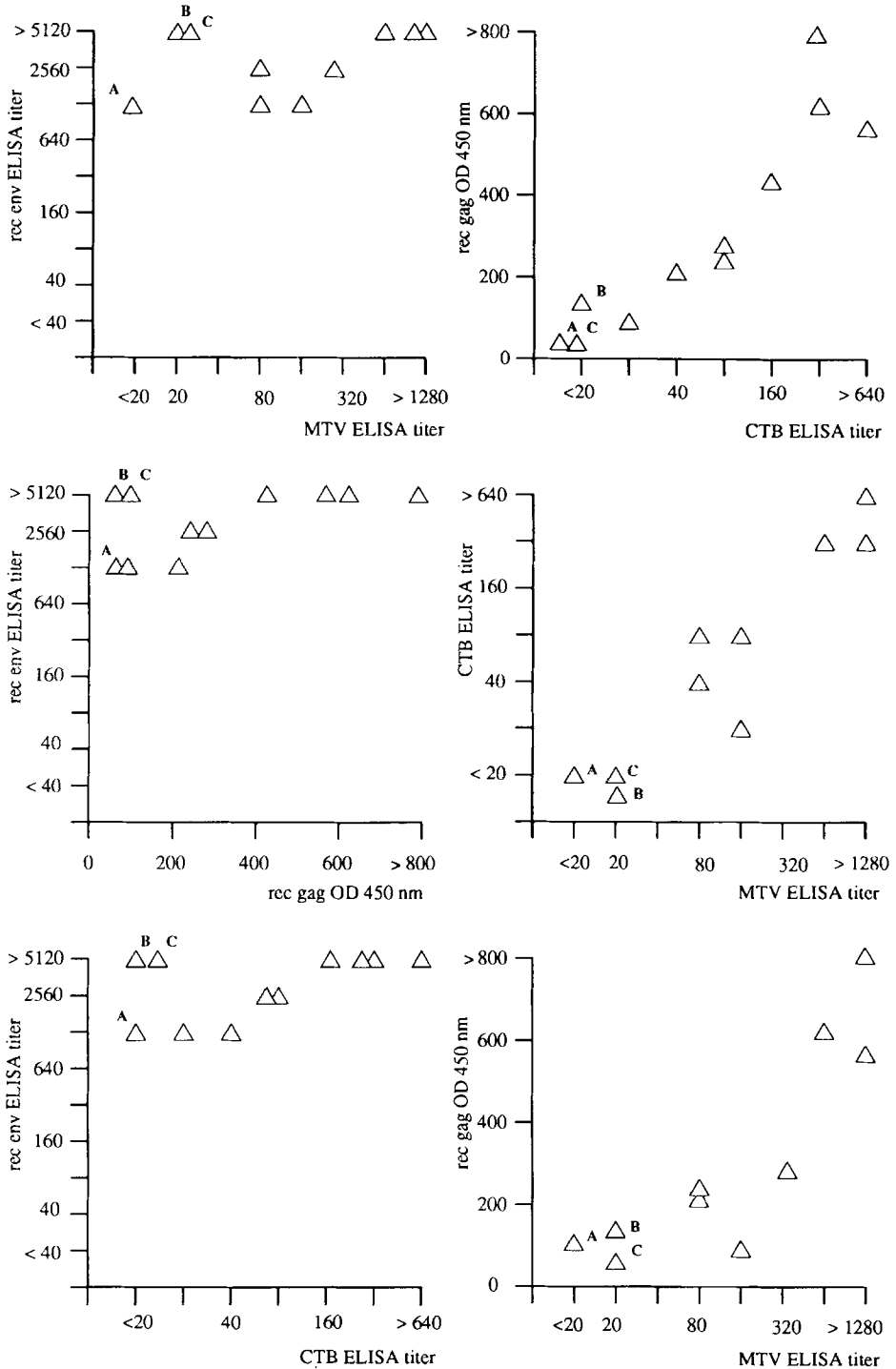
Fig. 3. Comparison of FIV-specific antibody levels in sera from naturally infected cats (n-11) obtained in the rec.*env*- and MTV-ELISAs, the rec.*env*- and rec.*gag*-ELISAs, the rec.*env*- and CTB-ELISAs, the rec.*gag*- and the CTB-ELISAs, the CTB- and the MTV-ELISAs and the rec.*gag*- and MTV-ELISAs. (For A, B, C: see Results).

infected with the progeny of infectious molecular clone (19K1), the development of serum antibodies to whole virus, FIV *env* and FIV *gag* proteins followed similar kinetics (Fig. 2a). Immediately after the onset of *env*-specific response detected in the rec.*env*-ELISA which was found within three weeks post infection and led to titres >5120 within five weeks, FIV *gag* protein-specific antibodies, measured in the rec.*gag*-ELISA and in the CTB-ELISA were detected. These increased rapidly during the subsequent weeks. In cat #13 the antibody response to FIV *gag* protein seemed to develop slightly later. Antibodies measured in the TV- and MTV-ELISAs developed concomittently with the *env*-specific antibodies in these four cats. A relatively late development of FIV *gag* protein-specific serum antibodies was more pronounced in the two SPF cats (#5 and #6) infected intravenously with the CrFK-cell adapted Petaluma strain of FIV (Fig. 2b). In cat #5 no FIV *gag* protein-specific antibodies could be detected within eight weeks post infection, whereas in cat #6 FIV *gag* protein-specific antibody titres slowly increased from five weeks post infection onward. These data coincided with the development of FIV *gag* protein-specific antibodies detectable in Western blot analysis in these two cats (data not shown). The development of *env*-specific serum antibodies did exhibit kinetics similar to those observed in the other four cats. Serum antibodies measured in the TV- and MTV-ELISAs in cat #6 were also similar to those of the other four cats, whereas the development of these antibodies in cat #5 again proved to be slower.

FIV-specific antibodies in clinically healthy and clinically suspected cats

Privately owned Dutch cats (n = 37) with (n = 11; positive in the TV-ELISA; Petcheck, IDEXX Corporation) and without (n = 26; negative in the TV-ELISA) clinical signs indicative for FIV infection, were tested for the presence of FIV-specific serum antibodies in the four newly developed ELISAs: MTV-, rec.*env*-, rec.*gag*-and CTB-ELISAs (Fig. 3).

None of the serum samples from cats without clinical signs scored positive in any of the newly developed ELISAs. The 11 cats with clinical signs indicative for feline AIDS, all scored positive in the rec.*env*-ELISA, with titres ranging from 320 to ⩾5120. Of the 11 positive samples three (samples A, B and C) scored consistently negative in the rec.*gag*-and the CTB-ELISA, and negative or low titred (titre = 20) in the MTV-ELISA. There proved to be an overall relation between the values found with the individual serum samples in the MTV-, rec.*gag*- and CTB-ELISAs which was not the case when the results of these assays were compared with those obtained in the more sensitive rec.*env*-ELISA.

Discussion

In this paper we have described four newly developed ELISAs based on different principles using recombinant FIV proteins or *gag* protein-specific monoclonal antibodies. New generations of diagnostic ELISAs are needed since the presently used classical assays, based on whole virus preparations have been shown to score too many false positives (Siebelink et al., 1990b; Reid et al., 1991). Furthermore, ELISAs based on this principle do not allow the identification of antibodies directed against individual viral proteins, which as in the case of HIV infection of humans may be of major importance for studies concerning the pathogenesis of the disease (Lange et al., 1986a; Allain et al., 1986; Allain et al., 1987;

Weber et al., 1987). All except the CTB-ELISA were based on the use of antigen coated plates to quantitate FIV *gag-* or *env*-specific antibodies in feline sera. With sera from experimentally infected cats it was shown that, as in HIV-1 infections in man (Saah et al., 1987; Race et al., 1991), *env*-specific antibodies were detected before the appearance of *gag*-specific antibodies. This difference was most pronounced in cats infected intravenously with the CrFK adapted Petaluma strain of FIV. The overall slower development of FIV-specific antibodies in these two cats as compared to those infected with the Dutch isolate Adam 19 or the molecular clone 19K1 indicates that either strain differences or adaptation of the virus to CrFK cells may directly influence the initial replication rate of the virus. The relatively slow development of *gag*-specific antibodies in the cats infected with the Petaluma strain was confirmed by showing that these findings coincided well with the development of antibodies reactive with p24 in the Western blot assay (not shown). After having monitored the development of serum antibodies, detectable with these assays in the experimentally infected cats during the first months after infection, their value for the detection of naturally infected domestic cats was studied by testing the serum samples of 11 cats suspected of suffering from FIV infection on basis of clinical signs and subsequent positive serology in the Petcheck ELISA. All the 26 clinically healthy cats scored negative when tested in all the newly developed serological tests. Among the 11 TV-ELISA positive cats, all scored positive in the rec.*env*-ELISA. Of these, three (samples A, B and C) were negative or borderline positive in the ELISAs detecting antibodies directed to the FIV *gag* protein. From cats A and C PBMC could be obtained, from which FIV was isolated (data not shown). This indicates that results found in the rec.*env*-ELISA are indeed confirmatory for FIV infection and that the other three ELISAs may fail to identify some FIV infected cats.

Although the envelope glycoprotein is subject to antigenic variation which may result in lack of reactivity with serum antibodies of cats infected with heterologous virus, no evidence was provided with the serum samples tested that this was the case. However, the combination of serological assays detecting both FIV *env-* and *gag*-specific antibodies, circumvents this possible problem and should be considered confirmative for FIV infection.

For the detection of *gag*-specific antibodies, the use of the MTV-ELISA based on FIV *gag*-specific monoclonal antibodies may be less suitable since agglomerates of different virus proteins may be bound to the plates. The results obtained with sera from experimentally infected (#5 and #6) cats suggest that this is indeed the case (Fig. 2).

A discrepancy in the presence of *env-* vs. *gag*-specific antibodies, as was shown in the early stages of infection in the experimentally infected cats #5 and #6, may cause false negative serological results when a system is used that does not detect *env*-specific antibodies. Such discrepant results may perhaps also be expected when cats progress towards AIDS, as has been documented in HIV symptomatic infections in man, in which *gag*-specific antibodies, either due to immune complex formation or loss of functional B lymphocytes, decline while *env*-specific antibodies persist (Lange et al., 1986a,b; 1987; Weber et al., 1987; McHugh et al., 1988; Teeuwsen et al., 1991). The discrepancies found in FIV *gag*-vs. *env*-specific antibodies in cats A, B and C may be a reflection of this phenomenon since they all exhibited serious signs of immunodeficiency at the time of sampling. Since a small number of animals has been tested, these ELISA systems should be used to test more cats in different stages of disease to assess that these phenomenons indeed play a role in FIV

pathogenesis. The documented failure of serological assays to identify "silently" FIV infected cats (Hopper et al., 1989), may perhaps be attributed to their inability to detect FIV *env* protein-specific antibodies. A similar observation has been described for certain HIV infections which initially were believed not to induce serum antibodies (Saah et al., 1987; Race et al., 1991).

In conclusion the data presented indicate that ELISAs based on the simultaneous detection of FIV *env* and *gag* protein-specific serum antibodies are highly specific and sensitive tools for the serology of FIV infection in cats. They also allow detailed studies of mechanisms underlying certain kinetics of the FIV-specific antibody response that have been documented also in HIV infections of humans.

Acknowledgements

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