JOURNAL OF VIROLOGY, Nov. 1995, p. 7371–7374 Vol. 69, No. 11
0022-538X/95/$04.00+0

Isolation of a Highly Cytopathic Lentivirus from a Nondomestic Cat

MARGARET C. BARR,[1]* LILY ZOU,[1] DONALD L. HOLZSCHU,[1] LINDSAY PHILLIPS,[2]†
FRED W. SCOTT,[1,3] JAMES W. CASEY,[1] AND ROGER J. AVERY[1]

Department of Microbiology and Immunology[1] and Cornell Feline Health Center,[3] College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, and Chicago Zoological Park, Brookfield, Illinois 60513[2]

Received 10 April 1995/Accepted 28 July 1995

A feline immunodeficiency virus-like virus (FIV-Oma) isolated from a Pallas' cat (*Otocolobus manul*) is highly cytopathic in CrFK cells, in contrast to the chronic, noncytolytic infection established by an FIV isolate from a domestic cat (FIV-Fca). The virions have typical lentivirus morphology, density, and magnesium-dependent reverse transcriptase activity. The major core protein is antigenically cross-reactive with that of FIV-Fca; however, FIV-Oma transcripts do not cross-hybridize with FIV-Fca. A conserved region of the FIV-Oma *pol* gene has 76 to 80% nucleic acid identity with the corresponding *pol* regions of other feline lentiviruses and 64 to 69% identity with those of human, ovine, and equine lentiviruses.

Feline immunodeficiency virus (FIV) infection in domestic cats is emerging as a useful laboratory model for human AIDS (2, 10, 33, 34). Under natural conditions, cats experience an asymptomatic carrier state for years following initial FIV infection before developing an AIDS-like disease (17). Cats experimentally infected with FIV exhibit signs of acute infection which resolve over a few months (36). The cats then become clinically asymptomatic; however, subclinical immune dysfunction occurs and becomes progressively more profound in cats with long-term infections (1, 2). Specific-pathogen-free cats in laboratory environments usually do not develop typical feline AIDS, but neurologic disease, B-cell lymphomas, and myelodysplasia have been observed (11, 37).

Among human immunodeficiency virus (HIV) and simian immunodeficiency virus isolates, significant strain differences have an impact on the development of diagnostic procedures, therapeutic agents, and vaccines (12, 15, 27). Likewise, FIV isolates from domestic and nondomestic cats exhibit heterogeneity at both the cellular and molecular levels (22, 23, 30). Regions of the FIV *pol* gene from several nondomestic cats have been amplified by PCR (6, 26), and one puma lentivirus has been fully sequenced (19); however, the full extent of biological and molecular diversity among the feline lentiviruses is unknown.

Here we report the isolation and preliminary characterization of a highly cytopathic lentivirus from a young adult male Pallas' cat (*Otocolobus manul*), which was imported into the United States with three other Pallas' cats. During quarantine, the cat tested positive for FIV with an enzyme-linked immunosorbent assay (ELISA) (PetChek FIV Antibody Test Kit; IDEXX Corp.) (24). On immunoblot analysis (4, 8, 35), the Pallas' cat's serum reacted with the major core protein, p24, of the prototype domestic cat isolate, FIV-Fca (Petaluma) (29). The other Pallas' cats tested negative for FIV in both antibody assays. Hematological values for the seropositive Pallas' cat were within normal ranges, and the cat appeared clinically normal; however, the cat was infected with a *Trypanosoma* species and *Hepatozoon canis* (5). In addition, the FIV-positive Pallas' cat's $CD4^+/CD8^+$ T-cell ratio (0.34) was substantially lower than those of the three seronegative Pallas' cats (mean = 2.12) (17a).

A peripheral blood mononuclear cell (PBMC) culture (29) from the Pallas' cat was stimulated by concanavalin A (5 μg/ml; Sigma) for 3 days and then maintained in the presence of human recombinant interleukin-2 (300 IU/ml; Cetus Corp.). Fresh interleukin-2-stimulated PBMCs from uninfected domestic cats were added on days 3 and 10 of cultivation. Magnesium-dependent reverse transcriptase (RT) activity (16) was detected in the supernatant of the Pallas' cat PBMC culture following 5 days of cocultivation with domestic cat PBMCs. Cytopathic effects consisted of ballooning degeneration and lysis of the cells; occasional small syncytia (fewer than five nuclei) were observed in cytospin preparations.

Supernatants from PBMC cultures exhibiting high levels of magnesium-dependent RT activity were filtered through a 0.22-μm-pore-size filter, inoculated onto Crandell feline kidney (CrFK) cells at 50% confluency and cultured in the presence of complete CrFK growth medium (minimal essential medium with 20% Leibovitz's L-15 medium, 4 mM L-glutamine, 1% gentamicin, and 5% fetal bovine serum). Numerous small syncytia were observed 3 to 4 days after inoculation of CrFK cells with Pallas' cat PBMC culture supernatant. Following passage of the CrFK cells, numerous large syncytia (>20 nuclei) and extensive cellular vacuolation were detected at confluency (Fig. 1a); the monolayer was completely lysed 4 to 5 days after passage. The virus (FIV-Oma) maintained this degree of cytopathicity over eight subsequent passages through CrFK cells. In contrast, CrFK cells chronically infected with FIV-Fca contained few small syncytia and had no apparent cytolysis (Fig. 1b). Typical lentivirus particles (110 to 130 nm in length) were seen by electron microscopy of thin sections of the infected CrFK cells (Fig. 1c). Virion density on a 5 to 20% sucrose gradient (Fig. 2) was determined to be approximately 1.16 g/cm^3. Virus-associated RT showed a strong preference for magnesium over manganese (Fig. 2).

Three FIV-Oma proteins with molecular masses of 10, 15, and 24 kDa, presumably encoded by *gag*, were detected by Western blotting (immunoblotting) of pelleted virus with the homologous serum at a 1:20 dilution (Fig. 3, lane 5). Additional proteins with molecular masses of 38, 48, and 50 kDa

* Corresponding author. Mailing address: Dept. of Microbiology and Immunology, VRT T7015, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853. Phone: (607) 253-3407. Fax: (607) 253-3419. Electronic mail address: mcb10@cornell.edu.

† Present address: University of California—Davis, Davis, CA 95616.

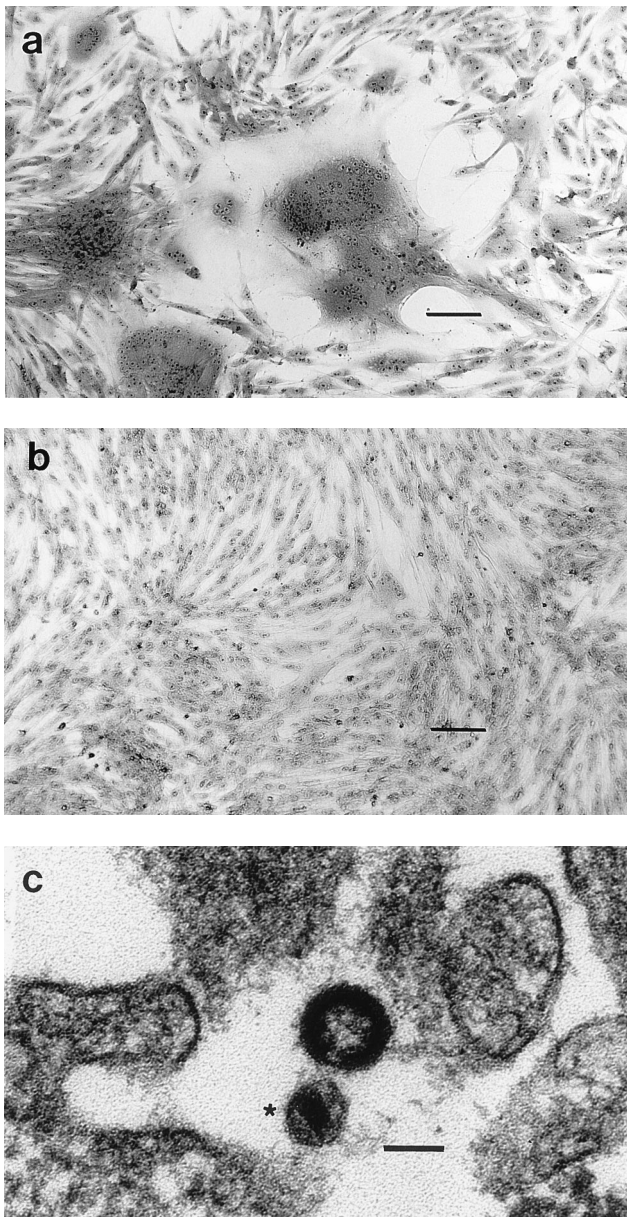

FIG. 1. (a and b) Crystal violet-stained CrFK cell monolayers infected with FIV-Oma (a) or FIV-Fca (Petaluma) (b). Large syncytia can be seen in the FIV-Oma-infected cells. Bars, 150 μm. (c) Electron micrograph of a thin section of CrFK cells infected with FIV-Oma. A mature virion (*) with the typical lentiviral cone-shaped electron-dense core structure is visible. The larger particle above it appears to be an immature virion. Bar, 100 nm.

were detected on Western blots with cell lysate preparations (not shown). The core antigens of FIV-Oma and FIV-Fca were reciprocally but incompletely cross-reactive. Antiserum against FIV-Fca showed no reactivity with the matrix (p15) and nucleocapsid (p10) proteins of FIV-Oma (Fig. 3, lane 6), although reactivity was strong against the homologous virus (Fig. 3, lane 3). Likewise, the FIV-Oma antiserum did not react with FIV-Fca p15 and p10 (Fig. 3, lane 2). Antiserum against feline syncytium-forming virus (FeSFV) did not recognize any FIV-Oma-associated proteins (Fig. 3, lane 7), and the Pallas' cat's serum did not cross-react with proteins of an isolate of FeSFV (not shown).

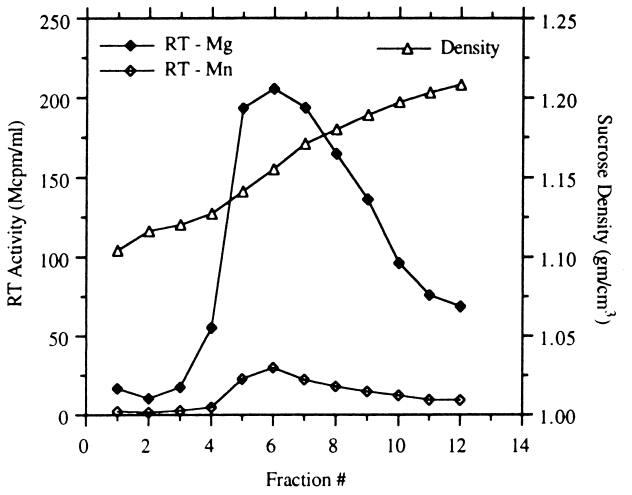

FIG. 2. Sucrose gradient purification of FIV-Oma. The volume of each fraction was 0.4 ml. RT-Mg, magnesium-associated RT activity; and RT-Mn, manganese-associated RT activity.

Viral RNA was extracted and purified from sucrose gradient-purified, pelleted virus (32), and radiolabeled cDNA was reverse transcribed with 200 ng viral RNA in RT buffer containing calf thymus random primers, deoxynucleoside triphosphates with [^{32}P]dCTP, and Moloney murine leukemia virus RT. Total RNA was extracted from FIV-infected and uninfected CrFK cells with acid guanidinium thiocyanate-phenol-chloroform (9) and was poly(A)$^+$ RNA selected with an oligo(dT) column (32). Transcripts were visualized by Northern (RNA) blot hybridization (13) with the FIV-Oma ^{32}P-cDNA or with ^{32}P-radiolabeled fragments of an FIV-Fca clone,

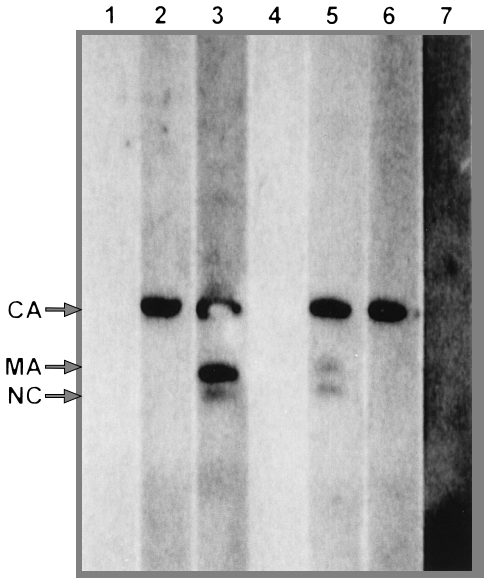

FIG. 3. Immunoblot analysis of FIV *gag*-encoded proteins reacted against serum from an uninfected cat (lanes 1 and 4), FIV-Oma antiserum (lanes 2 and 5), FIV-Fca (Petaluma) antiserum (lanes 3 and 6), or FeSFV antiserum (lane 7). Lanes 1 to 3, FIV-Fca proteins (1.4 μg per lane); lanes 4 to 7, FIV-Oma proteins (1.4 μg per lane). The white spot in the CA band on lane 3 is an "antiband" which can result from depletion of the chemiluminescence substrate at the site of excess antigen or antibody. CA, capsid protein (p24); MA, matrix protein (p15); NC, nucleocapsid protein (p10).

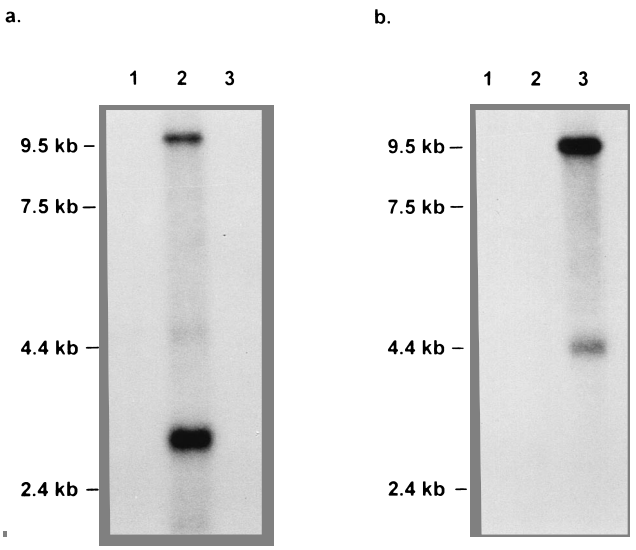

FIG. 4. Northern blot of poly(A)$^+$-selected RNA hybridized to a ^{32}P-labeled FIV-Oma virion cDNA probe (a) or to ^{32}P-labeled FIV-14 *Nhe*I fragments (which include most of the FIV-14 genome) (b). Lanes 1, RNA (3.0 μg) from uninfected CrFK cells; lanes 2, RNA (1.5 μg) from FIV-Oma-infected CrFK cells; lane 3, RNA (1.0 μg) from CrFK cells chronically infected with FIV-Fca (Petaluma). The exposure time was 12 h.

FIV-14 (25). An RNA species of about 10.0 kb, presumably genomic-length RNA, was detected by the homologous cDNA probe (Fig. 4a, lane 2). Additional RNA species of about 4.5, 3.0, and 1.5 to 2.0 kb were also detected, although the smallest transcripts were only faintly visible under these exposure conditions. FIV-Fca sequences in RNA from FIV-Fca-infected CrFK cells were not evident with this probe (Fig. 4a, lane 3). After the blot was stripped, FIV-14 probes detected viral transcripts in poly(A)$^+$ RNA from FIV-Fca-infected CrFK cells (Fig. 4b, lane 3) but showed no cross-hybridization with FIV-Oma-specific transcripts (Fig. 4b, lane 2).

PCR was performed on FIV-Oma cDNA with commercially available reagents (GeneAmp; Perkin-Elmer Cetus) and standard techniques (31). The sample was subjected to a 4-min heat shock step at 94°C, 10 cycles of amplification (94°C for 1 min, 37°C for 2 min, and 72°C for 3 min), 30 more cycles of amplification (94°C for 30 s, 55°C for 1 min, and 72°C for 1 min), and a final cycle of 72°C for 5 min. Degenerate lentivirus *pol* gene primers (14) allowed amplification of a 437-bp fragment, which was then ligated into the pBluescript SK− phagemid cloning vector (Stratagene). The nucleic acid sequence for the 381 bases of the FIV-Oma *pol* fragment internal to the PCR primer sequences was determined with an automated DNA sequencer (model 373A; Applied Biosystems Inc.) and confirmed by conventional chain termination sequencing (Sequenase version 2.0; United States Biochemical). NCBI Blast (GenBank) and MegAlign (DNAStar, Inc.) were used to determine nucleic acid identities with FIV-Fca isolates (79 to 80%), puma lentiviruses (79 to 80%), lion lentiviruses (76 to 80%), equine infectious anemia virus (69%), visna virus (64%), and HIV type 1 (HIV-1) (64%).

In this paper, we have presented direct evidence of a unique lentivirus infection in the endangered Pallas' cat. Presumptive FIV infection of nondomestic cats, based on detection of antibodies to FIV, has been reported previously (3, 21, 26), and FIV-like lentiviruses have been isolated from pumas (26) and a lion (6). About 80% of the lions in Kruger National Park in South Africa are FIV antibody positive, yet these cats appear clinically normal (26). In this respect, feline lentivirus infections in some species of the family Felidae may be similar to nonpathogenic simian immunodeficiency virus infections in some nonhuman primates. The pathogenic potential of FIV-Oma in the infected Pallas' cat is unclear. The cat appears healthy; however, the *Trypanosoma* and *Hepatozoon* parasitic infections may indicate marginally depressed immune function. The cat's low CD4$^+$/CD8$^+$ T-cell ratio also suggests subclinical immunosuppression. Disease may occur as the infection progresses and CD4$^+$ T-cell levels further decline.

The antigenic differences between FIV-Fca (Petaluma) and FIV-Oma detected by immunoblot analysis indicate some divergence between these viruses. Although the major *gag* protein (capsid or p24) of FIV-Oma and FIV-Fca is recognized by antiserum to either virus, antigenic cross-reactivity is poor or absent for the minor *gag* proteins (nucleocapsid or p10 and matrix or p15). The lack of cross-hybridization on Northern blots and the sequence data for the *pol* PCR fragment also indicate substantial divergence between the Pallas' cat isolate and other feline lentiviruses. Further cloning and sequencing of the FIV-Oma genome will allow a more comprehensive determination of the genetic relatedness of this virus to other feline lentiviruses.

The aggressive growth, cytopathicity, and tropism of FIV-Oma in CrFK cells are unlike those of other feline isolates. Some domestic cat strains of FIV, including FIV-Fca (Petaluma) and our FIV-Fca (Augusta) isolate (2a), establish chronic infections in CrFK cells with little or no cytopathic effect (37). Other strains appear to infect only feline PBMCs or T-lymphoblastoid-like cell lines (22, 30). Acute, lytic infection of CrFK cells with extensive syncytium formation, as seen with FIV-Oma, has not been reported for any of the domestic cat FIV isolates (22, 28, 30, 37). Syncytium formation and lysis in non-T-cell cultures have been described, however, for some ovine and caprine lentiviruses (7). In addition, differences in the degree of cell killing and syncytium formation in T-cell lines have been reported for HIV-1 and HIV-2 isolates (20).

Because the growth of FIV-Oma in CrFK cells resembles that of FeSFV, which is commonly isolated from domestic cat tissues (18, 28), the cytopathic effects observed were first considered to be due to FeSFV infection. However, FeSFV antigens are not detectable by immunoblot analysis of FIV-Oma-associated proteins, the RT of FIV-Oma exhibits a dependence on magnesium rather than the dependence on manganese which is characteristic of FeSFV isolates, and FIV-Oma virion morphology is typical of a lentivirus. Finally, the sequence data for the *pol* gene fragment position FIV-Oma with other lentiviruses.

The intriguing differences between the cytopathicity and tropism of FIV-Oma and the cytopathicity and tropism of FIV-Fca provide an opportunity to pinpoint the genetic region(s) responsible for these effects. The acutely cytopathic infection of CrFK cells by FIV-Oma may also provide a tissue culture system in which effects of antilentiviral therapeutic agents can be readily assayed. The infectivity and pathogenic potential of this isolate in domestic cats must still be explored; however, FIV-Oma and recombinants of domestic cat FIV isolates may ultimately enhance FIV infection of cats as an animal model for AIDS research.

Nucleotide sequence accession number. The nucleotide sequence determined in this study has been deposited in the GenBank sequence database under accession number U31349.

We thank N. C. Pedersen (University of California, Davis) for supplying the FIV-Fca (Petaluma) isolate and the antisera to FeSFV. We acknowledge the NIH AIDS Research and Reference Reagent Program for supplying the molecular clone FIV-14. We thank A.-L. Wil-

liamson (University of Cape Town Medical School, Rondebosch, South Africa) and J. Telford (Cornell University) for assistance with electron microscopy, M. Pough (New York State Diagnostic Laboratory) for performing the ELISAs, W. Hoose for technical support, and C. Poker for assistance with the photomicrographs.

This work was partially funded by the President's Council of Cornell Women and the Cornell Feline Health Center, and we are grateful for their support.

REFERENCES

1. **Ackley, C. D., J. K. Yamamoto, N. Levy, N. C. Pedersen, and M. Cooper.** 1990. Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. J. Virol. **64:**5652–5655.
2. **Barlough, J. E., C. D. Ackley, J. W. George, N. Levy, R. Acevedo, P. F. Moore, B. A. Rideout, M. D. Cooper, and N. C. Pedersen.** 1991. Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: comparison of short-term and long-term infections. J. Acquired Immune Defic. Syndr. **4:**219–227.

2a. **Barr, M.** Unpublished data.

3. **Barr, M. C., P. P. Calle, M. E. Roelke, and F. W. Scott.** 1989. Feline immunodeficiency virus infection in nondomestic felids. J. Zoo Wildl. Med. **20:**265–272.
4. **Barr, M. C., M. B. Pough, R. H. Jacobson, and F. W. Scott.** 1991. Comparison and interpretation of diagnostic tests for feline immunodeficiency virus infection. J. Am. Vet. Med. Assoc. **199:**1377–1381.
5. **Barr, S. C., D. D. Bowman, L. G. Phillips, and M. C. Barr.** 1993. *Trypanosoma manulis* n. sp. from a Pallas' cat (*Felis manul*). J. Eukaryot. Microbiol. **40:**233–237.
6. **Brown, E. W., N. Yuhki, C. Packer, and S. J. O'Brien.** 1994. A lion lentivirus related to feline immunodeficiency virus: epidemiologic and phylogenetic aspects. J. Virol. **68:**5953–5968.
7. **Campbell, B. J., D. Thompson, J. R. Williams, S. G. Campbell, and R. J. Avery.** 1993. Characterization of a New York ovine lentivirus isolate. J. Gen. Virol. **74:**201–210.
8. **Carlson, J. R., J. Yee, S. H. Hinrichs, M. L. Bryant, M. B. Gardner, and N. C. Pedersen.** 1987. Comparison of indirect immunofluorescence and Western blot for detection of anti-human immunodeficiency virus antibodies. J. Clin. Microbiol. **25:**494–497.
9. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
10. **Dow, S. W., M. L. Poss, and E. A. Hoover.** 1990. Feline immunodeficiency virus: a neurotropic lentivirus. J. Acquired Immune Defic. Syndr. **3:**658–668.
11. **English, R. V., P. Nelson, C. M. Johnson, M. Nasisse, W. A. Tompkins, and M. B. Tompkins.** 1994. Development of clinical disease in cats experimentally infected with feline immunodeficiency virus. J. Infect. Dis. **170:**543–552.
12. **Fomsgaard, A., V. M. Hirsch, J. S. Allan, and P. R. Johnson.** 1991. A highly divergent proviral DNA clone of SIV from a distinct species of African green monkey. Virology **182:**397–402.
13. **Fourney, R. M., J. Miyakoshi, R. S. Day III, and M. C. Paterson.** 1988. Northern blotting: efficient staining and transfer. Focus **10:**5–7.
14. **Gelman, I. H., J. Zhang, E. Hailman, H. Hanafusa, and S. S. Morse.** 1992. Identification and evaluation of new primer sets for the detection of lentivirus proviral DNA. AIDS Res. Hum. Retroviruses **8:**1981–1989.
15. **Hahn, B. H., G. M. Shaw, S. K. Arya, M. Popovic, R. C. Gallo, and F. Wong-Staal.** 1984. Molecular cloning and characterization of the HTLV-III virus associated with AIDS. Nature (London) **312:**166–169.
16. **Heine, C., D. C. Kelly, and R. J. Avery.** 1980. The detection of intracellular retrovirus-like entities in *Drosophila melanogaster* cell cultures. J. Gen. Virol. **49:**385.
17. **Ishida, T., and I. Tomoda.** 1990. Clinical staging of feline immunodeficiency virus infection. Jpn. J. Vet. Sci. **52:**645–648.

17a. **Kennedy-Stoskopf, S. (North Carolina State University).** Personal communication.

18. **Kukedi, A., A. Bartha, and B. Nagy.** 1988. Latent infection with feline syncytial virus of cell cultures prepared from the kidneys of newborn kittens. Vet. Microbiol. **16:**9–14.
19. **Langley, R. J., V. M. Hirsch, S. J. O'Brien, D. Adger-Johnson, R. M. Goeken, and R. A. Olmsted.** 1994. Nucleotide sequence analysis of a puma lentivirus (PLV-14): genomic organization and relationship to other lentiviruses. Virology **202:**853–864.
20. **Levy, J. A.** 1993. Pathogenesis of human immunodeficiency virus infection. Microbiol. Rev. **57:**183–289.
21. **Lutz, H., E. Isenbugel, R. Lehmann, R. H. Sabapara, and C. Wolfensberger.** 1992. Retrovirus infections in non-domestic felids: serologic studies and attempts to isolate a lentivirus. Vet. Immunol. Immunopathol. **35:**215–224.
22. **Miyazawa, T., M. Fukasawa, A. Hasegawa, N. Maki, K. Ikuta, E. Takahashi, M. Hayami, and T. Mikami.** 1991. Molecular cloning of a novel isolate of feline immunodeficiency virus biologically and genetically different from the original U.S. isolate. J. Virol. **65:**1572–1577.
23. **Miyazawa, T., K. Tomonaga, Y. Kawaguchi, and T. Mikami.** 1994. The genome of feline immunodeficiency virus. Arch. Virol. **134:**221–234.
24. **O'Connor, T. P., Jr., S. Tanguay, R. Steinman, R. Smith, M. C. Barr, J. K. Yamamoto, N. C. Pedersen, P. R. Andersen, and Q. J. Tonelli.** 1989. Development and evaluation of immunoassay for detection of antibodies to the feline T-lymphotropic lentivirus (feline immunodeficiency virus). J. Clin. Microbiol. **27:**474–479.
25. **Olmsted, R. A., A. K. Barnes, J. K. Yamamoto, V. M. Hirsch, R. H. Purcell, and P. R. Johnson.** 1989. Molecular cloning of feline immunodeficiency virus. Proc. Natl. Acad. Sci. USA **86:**2448–2452.
26. **Olmsted, R. A., R. Langley, M. E. Roelke, R. M. Goeken, D. Adger-Johnson, J. P. Goff, J. P. Albert, C. Packer, M. K. Laurenson, T. M. Caro, L. Scheepers, D. E. Wildt, M. Bush, J. S. Martenson, and S. J. O'Brien.** 1992. Worldwide prevalence of lentivirus infection in wild feline species: epidemiologic and phylogenetic aspects. J. Virol. **66:**6008–6018.
27. **Oram, J. D., R. G. Downing, M. Roff, J. C. Clegg, D. Serwadda, and J. W. Carswell.** 1990. Nucleotide sequence of a Ugandan HIV-1 provirus reveals genetic diversity from other HIV-1 isolates. AIDS Res. Hum. Retroviruses **6:**1073–1078.
28. **Pedersen, N. C.** 1988. Feline syncytium-forming virus infection, p. 77–82. *In* P. W. Pratt (ed.), Feline infectious diseases. American Veterinary Publications, Inc., Goleta, Calif.
29. **Pedersen, N. C., E. W. Ho, M. L. Brown, and J. K. Yamamoto.** 1987. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science **235:**790–793.
30. **Phillips, T. R., R. L. Talbott, C. Lamont, S. Muir, K. Lovelace, and J. H. Elder.** 1990. Comparison of two host cell range variants of feline immunodeficiency virus. J. Virol. **64:**4605–4613.
31. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
32. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
33. **Shelton, G. H., C. K. Grant, S. M. Cotter, M. B. Gardner, W. D. Hardy, Jr., and R. F. DiGiacomo.** 1990. Feline immunodeficiency virus and feline leukemia virus infections and their relationship to lymphoid malignancies in cats: a retrospective study. J. Acquired Immune Defic. Syndr. **3:**623–630.
34. **Siebelink, K. H. J., I.-H. Chu, G. F. Rimmelzwaan, K. Weijer, R. van Herwijnen, P. Knell, H. F. Egberink, M. L. Bosch, and A. D. M. E. Osterhaus.** 1990. Feline immunodeficiency virus (FIV) infection in the cat as a model for HIV infection in man: FIV-induced impairment of immune function. AIDS Res. Hum. Retroviruses **6:**1373–1378.
35. **Towbin, H., T. Staehelin, and J. Gorden.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
36. **Yamamoto, J. K., N. C. Pedersen, E. W. Ho, T. Okuda, and G. H. Theilen.** 1988. Feline immunodeficiency syndrome—a comparison between feline T-lymphotropic lentivirus and feline leukemia virus. Leukemia **2:**204S–215S.
37. **Yamamoto, J. K., E. Sparger, E. W. Ho, P. R. Andersen, T. P. O'Connor, C. P. Mandell, L. Lowenstine, R. Munn, and N. C. Pedersen.** 1988. Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. Am. J. Vet. Res. **49:**1246–1258.