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Isolation and Partial Characterization of Infectious Molecular Clones of Feline Immunodeficiency Virus Obtained Directly from Bone Marrow DNA of a Naturally Infected Cat

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Replication-competent molecular clones of feline immunodeficiency virus (FIV) were isolated directly from the DNA of bone marrow cells of a naturally FIV-infected cat. After transfection in a feline kidney cell line (CrFK) and subsequent cocultivation with peripheral blood mononuclear cells (PBMC), the viral progeny of the clones was infectious for PBMC but not for CrFK cells. PBMC infected with these clones showed syncytium formation, a decrease in cell viability, and gradual loss of CD4$^+$ cells. The restriction maps of these clones differed from those obtained for previously described molecular clones of FIV derived from cats in the United States. The predicted amino acid sequence similarity of the envelope genes of the two clones was 99.3%, whereas the similarities of the sequences of the clones to those of two molecular clones from the United States, Petaluma and PPR, were 86 and 88%, respectively. Most of the differences between the amino acid sequences of the two clones and those of the clones from the United States were found in five different hypervariable (HV) regions, HV-1 through HV-5. The viral progeny of one of these clones was inoculated into two specific-pathogen-free cats. The animals seroconverted, and the virus could be reisolated from their PBMC.

Feline immunodeficiency virus (FIV) is a T-lymphotropic lentivirus initially isolated from an immunodeficient cat (23). FIV infection in cats can lead to immunological abnormalities similar to those seen in human immunodeficiency virus type 1 (HIV-1) infected humans, like a depletion of CD4$^+$ cells in circulation (2, 3, 31). Similarly, the peripheral blood mononuclear cells (PBMC) from FIV-infected cats show reduced proliferative responses to mitogens and to exogenous interleukin 2 (IL-2) in vitro (3, 13, 28). These similarities in biological behavior between HIV-1 and FIV may make FIV infection of cats a suitable small-animal system to study lentivirus pathogenesis as a model for human AIDS and may likewise identify the FIV system as a model for HIV vaccine development.

Lentiviruses display a large degree of molecular and biological variation. This variation is generally ascribed to the low fidelity of the viral enzyme reverse transcriptase (RT) in copying the viral genomic RNA to DNA (25, 26). The use of complete molecular clones allows one to obtain genomically homogeneous viral populations. Relating the biological properties of such populations to the genomic structure of the molecular clone from which they were derived may reveal the molecular basis of biological variation and may identify determinants of viral virulence. To date, molecular clones of three FIV strains have been described, two from the United States (the 34TF10 clone of the Petaluma strain [22, 30] and the PPR clone of the San Diego strain [24] and one from Japan (JM1 [21]), which were all obtained from the DNA of in vitro-propagated cells. Culturing in vitro may select for certain virus subpopulations and can induce new features in the propagated virus. Culturing of simian immunodeficiency virus (SIV) in human cells, e.g., can lead to the introduction of a premature termination codon in the viral transmembrane glycoprotein (14, 17). These problems make the availability of complete molecular clones obtained directly from the DNA of in vivo-infected cells highly desirable. Here we report on two replication-competent molecular clones derived directly from the DNA of bone marrow cells from an FIV-infected cat. The genomic structures of these clones and their presumed envelope gene sequences are presented.

HIV-1, HIV-2, and SIV infect cells via the CD4 molecule, which acts as the cellular receptor for these viruses (8, 15, 18, 19). These viruses are also cytopathic for CD4$^+$ cells in vitro, and this cytopathic effect may at least in part underlie the virally induced immunodeficiency characterized by CD4$^+$ cell depletion in vivo. The CD4 analog in cats has been identified recently (1), but its role in FIV binding and entry is not yet clear. FIV infection also results in a reduction of CD4$^+$ cells in circulation in infected cats. We have therefore investigated whether the molecular clones of FIV described here are cytopathic for CD4$^+$ cells in vitro.

MATERIALS AND METHODS

Cells and virus. Bone marrow cells were obtained from the femur of a 4-year-old naturally FIV-infected free-roaming cat (Amsterdam-19) suffering from a generally debilitating disease and intermittent disease symptoms, including respiratory infections and chronic diarrhea, all suggestive of an immunodeficiency syndrome (23, 32). The cells were washed twice and used for isolation of genomic DNA (see below).

PBMC were isolated from heparinized blood from the cat by Ficoll density gradient centrifugation. The PBMC were washed three times with RPMI 1640 (GIBCO), supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and β-mercaptoethanol (2 × 10^{-5}

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M), designated culture medium (CM). A total of 10^7 PBMC were stimulated with 5 μg of concanavalin A (ConA) (Flow Laboratories, Inc.) per ml in CM supplemented with 10% heat-inactivated fetal calf serum (FCS). After 3 days, the cells were washed once and further cultured with CM supplemented with 10% FCS and IL-2 (100 IU/ml) (Cetus). The culture supernatant was monitored weekly for the presence of FIV antigen in an FIV antigen capture enzyme-linked immunosorbent assay (ELISA) as described previously (29). A virus stock of FIV from cat Amsterdam-19 (designated FIV Amsterdam-19) was made from the supernatant of this culture 21 days after stimulation and stored in aliquots at −135°C. The RT activity was assayed and was 7.0 × 10^4 cpm/ml.

PBMC were derived from heparinized blood of a specific-pathogen-free (SPF) cat by Ficoll density gradient centrifugation. The cells were washed twice and frozen at −135°C in aliquots in CM supplemented with 10% FCS and 10% dimethyl sulfoxide. Before use, the cells were thawed and stimulated with ConA (5 μg/ml) for 3 days and further cultured with IL-2 (100 IU/ml).

An FIV-susceptible clone of the Crandell feline kidney cell line (CrFK) was obtained from N. Pedersen (32). The cells were cultured in Dulbecco's modified Eagle's medium supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), β-mercaptoethanol (2 × 10^{-5} M), and 10% FCS.

DNA isolation. Genomic DNA was isolated from bone marrow cells. A total of 10^8 bone marrow cells of cat Amsterdam-19 were washed twice and lysed with 100 μg of proteinase K per ml and 0.5% sodium dodecyl sulfate (SDS) for 16 h at 42°C. Cesium chloride (1.25 g/ml of lysate) was added, and after centrifugation for 44 h at 60,000 rpm in a 70 Ti rotor (Beckman), fractions containing the high-molecular-weight DNA were collected, pooled, and dialyzed against TE (10 mM Tris, pH 8.0, and 0.1 mM EDTA).

Southern blot analysis of genomic DNA. Genomic DNA was digested with *Bam*HI or *Nhe*I. After electrophoresis, the DNA was transferred to nitrocellulose and hybridized with the ^{32}P-labeled *gag* probe P_2 (see below) in hybridization buffer (3× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 50 mM Tris, 5× Denhardt's solution, 0.5% SDS, 5 mM EDTA, 50% formamide, 10% dextran sulfate, and 10 μg of salmon sperm DNA per ml). The blots were washed twice with 0.2× SSC–0.1% SDS for 30 min at 68°C each time and autoradiographed.

Molecular cloning of FIV proviral DNA. Genomic DNA from bone marrow cells from cat Amsterdam-19 was partially digested with *Sau*3A. After size fractionation on a 5 to 25% NaCl gradient, 10- to 20-kb fragments were ligated to EMBL-3 lambda arms (Stratagene) and packaged in vitro. The unamplified lambda phage library was screened with the probes P_2 (1.8-kb *Sst*I-*Bam*HI *gag* fragment) and P_{22} (2.1-kb *Bam*HI-*Bam*HI *pol* fragment), which were obtained from R. Olmsted (22), and ENV-4 (a 2.6-kb fragment spanning the complete envelope gene obtained by polymerase chain reaction [25a]).

Positive plaques were purified, and the DNA was isolated. *Sph*I-*Sal*I fragments of these clones were subcloned in pUC19. The subclones were used for the dideoxynucleotide chain termination sequence reaction (27) of the *env* gene. The nucleotide and protein alignments as well as the percentages of similarity were determined with Lasergene software (DNAstar Inc., London, United Kingdom).

Purified lambda DNA was used for the construction of a restriction enzyme map. Lambda DNA from the clones was digested with the restriction enzymes *Bam*HI, *Bgl*II, *Eco*RI, *Kpn*I, *Nhe*I, *Sph*I, and *Sst*I and, after electrophoresis on a 0.8% agarose gel, transferred to nitrocellulose. The filters were subsequently hybridized with P_2, P_{22}, or ENV-4 and autoradiographed.

DNA transfection. A sample of 5 μg of lambda DNA was transfected into CrFK cells by using the cationic lipid DOTMA (lipofectin; Bethesda Research Laboratories, Inc.) according to the protocol of the manufacturer. Briefly, 5 μg of DNA in 50 μl of H_2O was mixed with 50 μl of lipofectin and incubated for 15 min at room temperature. CrFK cells were washed with serum-free Dulbecco's modified Eagle's medium, and the lipofectin reagent-DNA complex was added. Twenty-four hours after transfection, CM containing 20% FCS, 100 IU of IL-2 per ml, and 5 × 10^6 ConA- and IL-2-stimulated PBMC from an SPF cat were added. After 72 h, the PBMC and CrFK cells were washed and cultured separately. The culture supernatant was monitored for the presence of FIV antigen in an FIV antigen capture ELISA. Eighteen days after transfection, a virus stock was made and stored in aliquots at −135°C. The RT activity was determined. The infected cells were used for electron microscopy essentially as described by Gelderblom et al. (11).

Infection of PBMC. ConA- and IL-2-stimulated PBMC of an SPF cat were infected with the molecular clones or with the biological isolate FIV Amsterdam-19 by resuspending 10^7 PBMC in 1 ml of virus dilution in CM containing 2 × 10^4 cpm of RT activity per ml. After 1 h at 37°C, the cells were washed twice with CM and cultured in CM supplemented with 10% FCS and 100 IU of IL-2 per ml. Twice a week, 1.5 ml of culture supernatant was centrifuged (10 min at 300 × *g*) and stored at −135°C until testing for the presence of FIV antigen and RT activity. The cells were monitored for cytopathic effects like syncytium formation and cell viability and for the percentages of $CD4^+$ and $CD8^+$ cells by fluorescence-activated cell sorter analysis with monoclonal antibodies provided by M. Cooper (1, 16).

RT assay. RT activity was assayed in a microassay as previously described by Gregersen et al. (12), with minor variations. Briefly, 1 ml of culture supernatant was precipitated with 0.25 ml of 32% polyethyleneglycol 6000–1.5 M NaCl. The pellets were resuspended in 10 μl of lysis buffer (50 mM Tris [pH 8.3], 20 mM dithiothreitol, 0.25% Triton X-100) and mixed with 40 μl of H_2O and 50 μl of RT cocktail (100 mM Tris [pH 7.9], 150 mM KCl, 10 mM $MgCl_2$, 4 mM dithiothreitol, 0.6 U of poly(rA) · oligo(dT), 60 μCi of [^{3}H]TTP per ml). After incubation at 37°C for 1 h, the DNA was precipitated with 20 μl of 120 mM $Na_4P_2O_7 \cdot 10H_2O$ in 60% trichloroacetic acid for 15 min at 4°C. The DNA was spotted onto glass fiber filters with a Skatron cell harvester and washed with 12 mM PPNa in 5% trichloroacetic acid. The filters were dried, and [^{3}H]TTP incorporation was measured in a β-scintillation counter.

Nucleotide sequence accession number. The sequences reported in this paper have been deposited in the GenBank data base (accession no. for clone 19k1 is M73964 and for clone 19k32 is M73965).

RESULTS

Molecular cloning and characterization of FIV provirus. Genomic DNA from bone marrow cells of a naturally FIV-infected free-roaming cat (Amsterdam-19) was digested with restriction enzymes and blotted onto nitrocellulose for hybridization with probe P_2, FIV-specific DNA could be detected in this DNA preparation as well as in the DNA

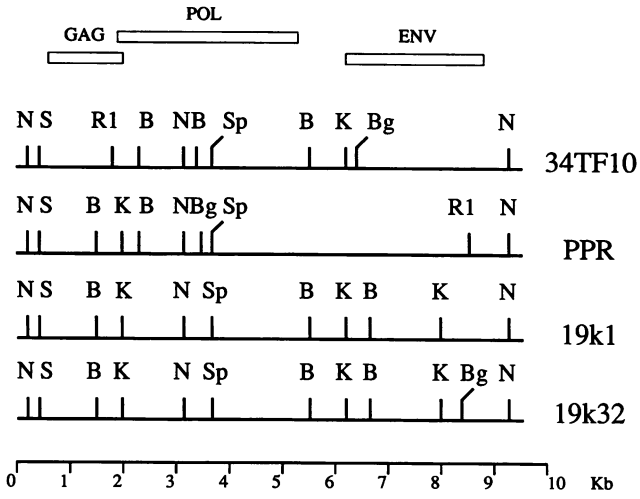

FIG. 1. Restriction map of infectious molecular clones of FIV strains Petaluma (34TF10), San Diego (PPR), and FIV Amsterdam-19 (19k1 and 19k32). Restriction enzyme maps of 34TF10 and PPR are as predicted from their published nucleotide sequences (24, 30). Maps of 19k1 and 19k32 were obtained by single and double restriction enzyme digestion of lambda DNAs which contain the full-length clones, followed by Southern blotting and hybridization with the ^{32}P-labeled FIV-specific probes P_2, P_{22}, or ENV-4. Restriction enzyme cleaving sites: B, *Bam*HI; Bg, *Bgl*II; R1, *Eco*RI; K, *Kpn*I; N, *Nhe*I; Sp, *Sph*I; and S, *Sst*I.

obtained from the spleen cells of cat Amsterdam-19 and the spleen and bone marrow cells of another naturally FIV-infected cat (data not shown). Digestion with *Bam*HI or *Nhe*I yielded internal FIV fragments with sizes of 3.8 and 3.2 kb, respectively. These fragments are also found in the maps of lambda clones 19k1 and 19k32 (see below).

Full-length proviral FIV clones were obtained from an EMBL-3 lambda phage library made directly from the DNA of the bone marrow cells of cat Amsterdam-19 by using the P_2, P_{22}, and ENV-4 probes for screening. The hybridizing clones were plaque purified and used for the isolation of lambda DNA. The replication competence of the cloned FIV provirus was assayed by transfection into CrFK cells (see below). Three clones (19k1, 19k32, and 19k36) gave rise to FIV antigen production upon transfection and were characterized further. Restriction maps of these three clones showed a high degree of similarity (Fig. 1). The maps of clones 19k1 and 19k36 were identical, whereas an additional *Bgl*II site is present in the envelope gene of clone 19k32. This additional *Bgl*II site was later confirmed by sequence analysis. When the predicted maps of the two U.S. clones (24, 30) were compared with that of clone 19k1, only 14 of 22 (34TF10 compared with 19k1, respectively) and 14 of 21 (PPR compared with 19k1, respectively) restriction sites appeared to be conserved. Between 34TF10 and PPR, 12 of 21 restriction sites are conserved (Fig. 1). This indicates a high degree of heterogeneity among these different FIV strains.

We obtained the nucleotide sequences of the envelope genes of the three clones of FIV Amsterdam-19. The nucleotide sequences of the envelope genes and the preliminary restriction site analyses of the 5′- and 3′-flanking sequences of clones 19k1 and 19k36 showed no differences, which suggests that these clones are fully identical. On the other hand, we found that clones 19k1 and 19k32 differed in the length of their flanking sequences (data not shown), and only the sequences of these clones were analyzed further. The 2,571 nucleotides of the envelope genes of these clones differ in only nine positions. Because of the lack of direct amino acid sequence data, it is as yet unclear where the exact initiation codon of the envelope gene is located. We have presumed the conserved ATG at position 6264 of clone 34TF10 of the Petaluma strain (30) to be the initiation codon for the *env* gene, which then encodes an 857-amino-acid glycoprotein with a potential cleavage site after 611 amino acids, resulting in a 611-amino-acid surface glycoprotein and a 246-amino-acid transmembrane glycoprotein. The predicted amino acid sequences of the envelope glycoproteins of these clones were compared with those of the 34TF10 clone of the Petaluma strain and the PPR clone of the San Diego strain (24, 30) (Fig. 2). The amino acid identity of the envelope glycoproteins of clones 19k1 and 19k32 is 99.3%. The sequence identities of these two highly related clones with the 34TF10 and PPR clone envelope sequences are 86 and 84%, respectively. We have aligned the four envelope gene sequences (Fig. 2). Although amino acid variation is found throughout the entire *env* gene, we observed the presence of five hypervariable (HV) regions, designated HV-1 through HV-5, as indicated in Fig. 2. These HV regions concur in part with the variable regions described by Phillips et al. (24). All four sequences are remarkably colinear, with insertions and/or deletions found only in HV-5 (Fig. 2). Most of the potential N-linked glycosylation sites are conserved between the envelope sequences of all four clones (21 of 22); some variation of the cysteine residues is observed within the first 150 amino acids.

Biological characterization. Lambda phage, containing apparently full-length proviral DNA of clones 19k1, 19k32, and 19k36, was transfected into CrFK cells. These were then cocultivated with PBMC for 72 h. Both cell populations were then cultured separately. In the PBMC cultures infected with clones 19k1 and 19k32, FIV antigen could be detected within 8 days after transfection. FIV antigen was also detected in the PBMC culture of clone 19k36 12 days after transfection. Transmission electron microscopy pictures from all of these cultures showed mature and immature virus particles and particles budding from the cell membrane (data not shown). FIV antigen could not be detected in the supernatant of the CrFK cell cultures. The PBMC cultures were expanded, and virus stocks were stored at −135°C. The RT activities of the stocks were 2.3×10^4, 2.9×10^4, and 0.5×10^4 cpm/ml for 19k1, 19k32, and 19k36, respectively. On the basis of apparent genetic identity of 19k1 and 19k36 (see above), we have concentrated on clones 19k1 and 19k32 for further analysis.

ConA- and IL-2-stimulated PBMC (10^7) from an SPF cat were infected with equal amounts of RT activity (2×10^4 cpm) of the viral progeny of clone 19k1 or 19k32 or the biological isolate FIV Amsterdam-19 obtained from in vitro-propagated PBMC. The cultures were monitored for syncytium formation, cell death, percent $CD4^+$ and $CD8^+$ cells, FIV antigen production, and RT activity. The results are shown in Fig. 3. Syncytium formation was seen 4 days postinfection (p.i.) in the culture infected with 19k1 and 6 days p.i. in the culture infected with 19k32 or the biological isolate (Fig. 4). FIV antigen and RT activity could be detected after 11 (19k1) and 14 (19k32 and FIV Amsterdam-19) days in the FIV-infected cultures but not in the noninfected control culture for 28 days p.i. (Fig. 3A and B).

The percent viable cells decreased from 95 to 55% during 28 days of culturing in the noninfected culture, whereas there was a more rapid decrease of viable cells in the

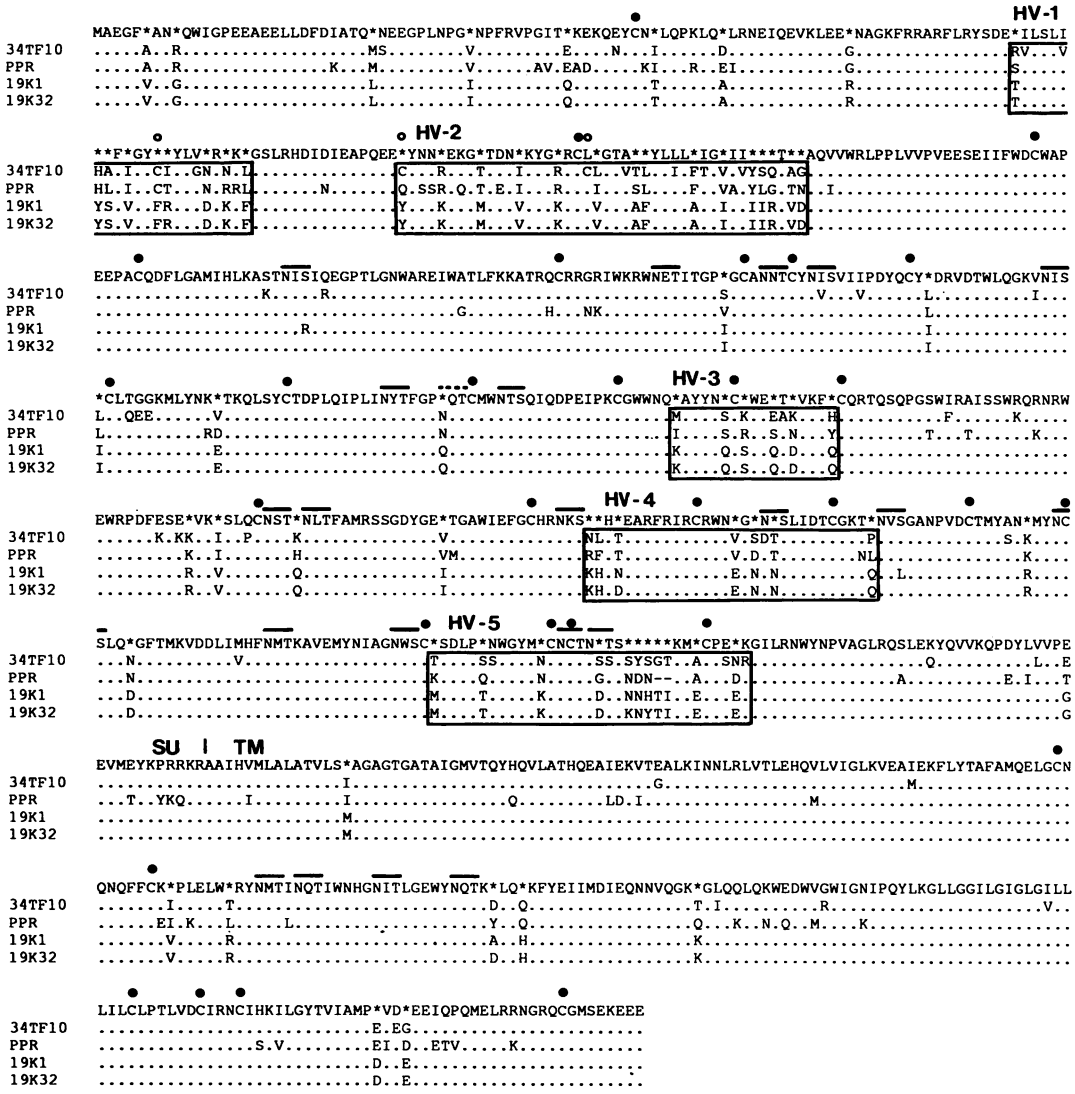

FIG. 2. Comparison of the predicted amino acid sequences of the envelope genes of molecular clones of the FIV strains Petaluma (34TF10), San Diego (PPR), and FIV Amsterdam-19 (19k1 and 19k32). HV regions (HV-1 through HV-5) are boxed. Solid lines, potential conserved glycosylation sites; dashed lines, nonconserved sites; closed circles, conserved cysteine residues; open circles, nonconserved residues. The presumed cleavage site between the surface glycoprotein (SU) and the transmembrane glycoprotein (TM) is also shown (see text for details).

FIV-infected cultures (only 15% viable cells after 28 days) (Fig. 3C), indicating cell death caused by FIV infection.

The percentages of the viable cells which were $CD4^+$ and $CD8^+$ cells could be measured accurately only during 18 days of culture following infection because of the low percent viable cells after this period. The percentages of viable cells which were $CD4^+$ and $CD8^+$ cells were 44 and 35%, respectively, at the time of infection. After 18 days of culture, these percentages in the noninfected culture were 36 and 38%, respectively. The mean percent $CD4^+$ cells in all of the infected cultures decreased to 6% in 18 days p.i. The mean percent $CD8^+$ cells increased to 66% in this period (data not shown), indicating a selective depletion of $CD4^+$ cells in infected cultures (Fig. 3D).

Two SPF cats were infected with the viral progeny of molecular clone 19k1, and two cats were infected with the biological isolate FIV Amsterdam-19. FIV could be isolated from these cats 2 weeks p.i., and seroconversion occurred 2 weeks later (data not shown). This demonstrates that these viruses are infectious in vivo.

DISCUSSION

We have generated molecular clones of FIV directly from the DNA of bone marrow cells of an FIV-infected cat and characterized these clones for genetic and biological properties. Cloning directly from in vivo-infected cells obviates the need for in vitro culturing of FIV in cell lines or in stimulated PBMC. In vitro culture systems may select some viral genotypes over others, as has been shown in numerous instances for HIV-1 (7, 9, 20), or may even introduce modifications in the cultured virus as demonstrated for

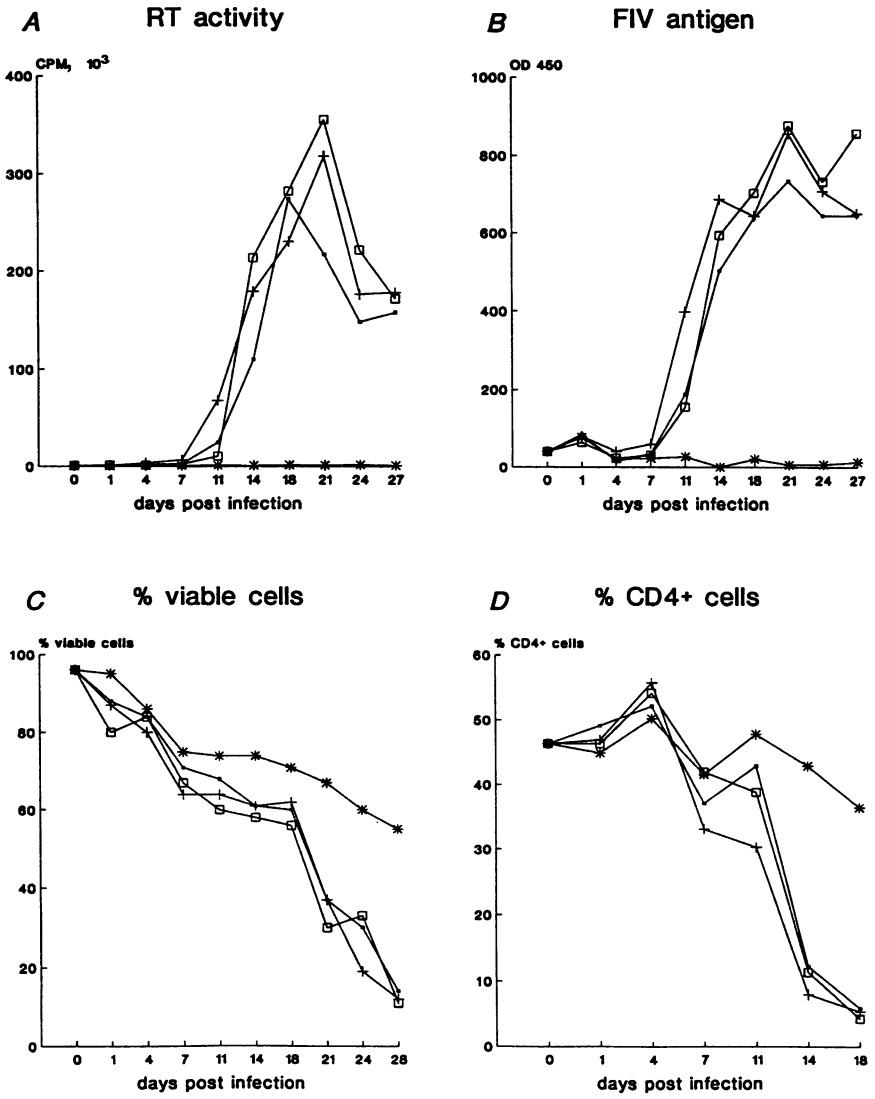

FIG. 3. Virus production, cell viability, and percent CD4$^+$ cells in feline PBMC infected in vitro with FIV as described in the text. (A) RT activities in culture supernatant; (B) FIV antigen in culture supernatant; (C) percent viable cells; (D) percentages of viable cells which were CD4$^+$. Symbols: ✳, noninfected; □, biological isolate FIV Amsterdam-19; ▪, molecular clone 19k1; +, molecular clone 19k32.

SIV_{mac} (14, 17). These drawbacks bring the inherent danger that a molecularly cloned virus obtained from in vitro cultures is not representative of the virus population present in the infected animal. The high load of FIV proviral DNA in the bone marrow cells of the naturally infected symptomatic cat Amsterdam-19 has allowed us to obtain complete molecular clones of FIV directly ex vivo. FIV-specific DNA could be detected in genomic DNA of bone marrow cells of cat Amsterdam-19 and in the DNA of bone marrow cells of only one of six other naturally infected cats. It is as yet unclear whether the detection of FIV proviral DNA in the genomic DNA of bone marrow cells of FIV-infected cats is a general feature. In this paper, we show that the viral progeny of these clones has biological properties very similar to the virus isolate obtained from the PBMC of cat Amsterdam-19.

FIV has been shown to cause a depletion of CD4-bearing cells in infected animals, as HIV-1 does in infected humans (2, 3, 31). For HIV-1, this depletion may be partly explained by its selective tropism for and cytopathic effect on CD4$^+$ cells, which has been demonstrated in vivo. Additionally, HIV-1 can downregulate the CD4 receptor on infected cells (5, 6). Although the CD4 analog in cats has been identified, the results as to its functioning as the receptor for FIV have so far been inconclusive. We demonstrate here that an FIV isolate as well as two infectious molecular clones of FIV selectively deplete CD4$^+$ cells from a culture of feline PBMC (Fig. 3D). The viruses also induce the formation of syncytia in these cultures, indicating that the observed CD4$^+$ cell depletion could well be the result of direct cytopathic effects, although we cannot rule out that downregulation of CD4 also plays a role. The fact that these molecularly cloned viruses induce syncytium formation and deplete CD4$^+$ cells in vitro may indicate that they do the same in vivo upon experimental infection. Such effects would result in a progression towards immunodeficiency. In preliminary experiments, the viral progeny of at least one of the two described clones (namely, 19k1) proved to be infectious in vivo. The experimentally infected animals will be monitored closely for

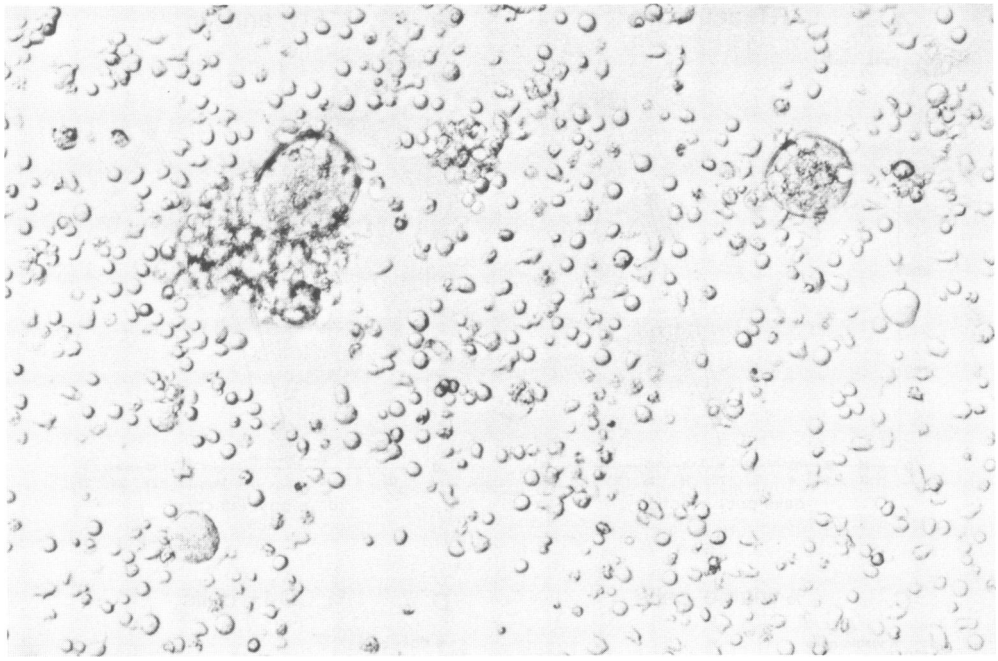

FIG. 4. Syncytium formation of feline PBMC which are infected with viral progeny of molecular clone 19k1 (magnification, ×400).

hematologic and immunologic parameters as well as for signs of FIV disease.

To facilitate transfection of the molecular clones, we have relied on an adherent cell line that is permissive for FIV replication, namely, the CrFK T-cell line. After transfection, the CrFK cells produced FIV particles whose viral progeny was thereupon rescued by cocultivation with feline PBMC. Interestingly, we could not show productive reinfection of CrFK cells by these viruses, indicating that one round of replication is not sufficient for the adaptation of FIV to CrFK cells. At the same time, these data demonstrate that the lack of growth of many FIV strains on CrFK cells is the result of a block at viral entry rather than at virus replication.

To date, two envelope sequences of FIV molecular clones have been obtained. The comparison of the sequences of these two U.S. isolates revealed an amino acid sequence similarity of approximately 85%. To assess the amount of variation between strains from widely different geographical locations, we have obtained the complete envelope sequences of the molecular clones 19k1 and 19k32. These two sequences are remarkably similar (99.3% similarity), whereas they differ by approximately 15% from both U.S. isolates, which is the amount of variation found between the U.S. isolates. A high degree of sequence similarity between two different proviruses is unusual for lentiviruses, which in general show much more sequence variation, especially in the envelope gene. The observed conservation may indicate that the obtained FIV clones are the progeny of the original viral clone that colonized the bone marrow of cat Amsterdam-19.

To predict the amino acid sequence of the envelope gene, we took the ATG at position 6264 of the 34TF10 clone of the Petaluma strain to be the start codon for *env*. This results in an 857-amino-acid glycoprotein with a potential cleavage site at position 611 (30). The precursor of the 857 amino acids can be cleaved into two smaller glycoproteins: an outer membrane protein of 611 amino acids and a transmembrane protein of 246 amino acids. The newly generated N terminus at the presumed transmembrane glycoprotein is highly hydrophobic and could serve as a fusion domain as demonstrated for the homologous regions in SIV_{mac} and HIV-1 (4, 10). Although there is no obvious sequence similarity between this domain in FIV and those of SIV and HIV-1, we observed a high incidence of amino acids with very short side chains like glycine and alanine in all three viruses. This GA repeat may well be important for the function of the fusion domain. Most of the structurally important features of the envelope glycoproteins, like cysteine residues and N-linked glycosylation sites, are conserved between these clones. Some nonconserved cysteine residues were found in the putative L gene region of the envelope glycoprotein, preceding the leader sequence identified by others (24, 30).

The molecular clones described in this report have been shown to be infectious both in vitro and in vivo. Such clones may enable us to delineate the molecular basis of the pathogenesis of FIV.

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