Feline Immunodeficiency Virus Infects both CD4⁺ and CD8⁺ T Lymphocytes

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Monoclonal populations of feline T cells, derived from a specific-pathogen-free cat and expressing either the CD4 or CD8 surface antigen, were infected in vitro with two geographically distinct isolates of feline immunodeficiency virus (FIV). Both infected T-cell subsets exhibited decreased cell viability, expressed FIV-encoded proteins, and generated reverse transcriptase activity. All clones examined retained their original surface phenotype after infection. It appears, therefore, that both CD4⁺ and CD8⁺ T cells may be productively infected by FIV in vivo.

Feline immunodeficiency virus (FIV) has been recognized as an important pathogen in areas throughout the world, including Europe, the United States, and Japan (8, 10, 25). FIV resembles other lentiviruses in morphology, protein structure, Mg²⁺ dependency of its reverse transcriptase (RT), and tropism for T lymphocytes (19). FIV is antigenically distinct from other animal lentiviruses, including human immunodeficiency virus (HIV) (23, 25). Nucleotide sequence analysis indicates that FIV is more closely related to ungulate than primate lentiviruses (23). However, the clinical signs of FIV-infected cats more closely resemble those attributable to HIV infection than those associated with ungulate lentiviruses.

The human CD4 antigen has been identified as the major natural receptor for HIV gp120 (6, 11, 14-16). Therefore, helper T lymphocytes and cells of the monocyte/macrophage lineage that express surface CD4 molecules are considered to be the primary targets for HIV infection (9). However, it has recently been shown that CD8⁺ CD4⁻ T cells can support replication of HIV and macaque simian immunodeficiency virus (SIV_{MAC}) (24). The cell surface receptor for FIV has not been identified; however, FIV infects and replicates in macrophages (5) and T lymphocytes (8, 10, 18, 19). Additionally, the Petaluma strain of FIV productively infects fibroblasts (25). It is not known whether FIV infection is restricted to cells expressing surface CD4 molecules. The recent availability of murine monoclonal antibodies with specificity for the homologs of CD4 (1) and CD8 (12) molecules in the cat has made it possible to establish the surface phenotype of feline T-cell subsets which can be infected by FIV. The studies described in this report were designed to determine the ability of FIV to infect biological clones of feline T cells expressing either the CD4 or the CD8 surface antigen.

Cloned T-cell lines were established from a specific-pathogen-free (SPF) cat (Liberty Laboratories, Liberty Corner, N.J.) that was serologically negative for FIV, feline syncytium-forming virus (FeSFV), and feline leukemia virus. Peripheral blood mononuclear cells (PBMC) obtained following venipuncture and Ficoll-sodium diatrizoate (His-

topaque-1077; Sigma, St. Louis, Mo.) density gradient centrifugation were stimulated with 5 µg of concanavalin A (Sigma) per ml for 3 days and then with 10% bovine T-cell growth factor (TCGF) (4) plus 25 U of human recombinant interleukin-2 (IL-2) (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml for 4 days. Lymphoblastoid cells were cloned by limiting dilution as described previously (4). Briefly, cells were seeded into round-bottom 96-well microtiter plates (Costar, Cambridge, Mass.) at an average of 1 or 0.3 cells per well with 5 × 10⁴ irradiated (3,000 rads) autologous PBMC, 10% bovine TCGF, and 25 U of IL-2 per ml in a final volume of 200 µl of complete RPMI. Complete RPMI consisted of RPMI 1640 medium containing 10% fetal bovine serum (Hyclone, Logan, Utah), 2 mM L-glutamine, 5 × 10⁻⁵ M 2-mercaptoethanol, and 50 µg of gentamicin per ml. Cloning frequencies for cells seeded at 1 and 0.3 cells per well were 21 and 15%, respectively. Clones were expanded sequentially to 48-well and 24-well plates (Costar), and the cell surface phenotypes were determined by indirect immunofluorescence and flow cytometry as described previously (13), using monoclonal antibodies specific for feline CD4 (1) and CD8 (12) determinants and fluorescein-labeled goat anti-immunoglobulins [affinity purified F(ab')₂ fragments; Cappel/Organon-Teknika Inc., Malvern, Pa.]. Of 26 expanded clones, 5 were CD4⁻ CD8⁺ and 21 were CD4⁺ CD8⁻. One of the CD8⁺ clones, designated 1F7, was subcloned as described above, and a selected subclone was designated 1F7.G6. The clones were maintained without feeder cells in complete RPMI with bovine TCGF and human IL-2, and aliquots were stored frozen in liquid nitrogen.

In three separate experiments, selected T-cell clones were infected in vitro with either the Petaluma isolate (19) obtained from the NIH/NIAID AIDS Research and Reference Reagent Program (catalog no. 236) or Peeper isolate (20) of FIV. The Peeper isolate was cloned by endpoint dilution and was free of either FeSFV or feline leukemia virus. Both isolates were propagated in concanavalin A-stimulated PBMC prepared from SPF cats or SPF cat-derived T-cell lines maintained in IL-2, and virus stocks were prepared from culture supernatants containing 1.2 × 10⁶ to 5 × 10⁶ cpm of viral RT activity per ml. T-cell clones were thawed and cultured for 9 to 20 days before infection with FIV. Cell

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FIG. 1. Immunofluorescent (EPICS flow cytometer) staining profiles of feline T-cell clones expressing either the CD4 (A) or CD8 (B) surface phenotype before (uninfected) or after in vitro infection with either the Petaluma or Peeper isolate of FIV. Negative profiles, for which only a secondary antibody was used, are indicated by the darkly shaded region of the curve.

surface phenotypes were determined before and at various days after infection (Fig. 1). For infection, 2×10^6 cells were incubated with 0.5 ml of virus stock for 30 min at 37°C . The cells were then resuspended in complete RPMI with bovine TCGF and human IL-2 and cultured in 1.5-ml cultures in 24-well plates at a density of 3.3×10^5 cells per ml. Control cultures of uninfected cells were also maintained under the same conditions. Every 2 or 3 days, the cells were centrifuged at $250 \times g$, supernatants were collected, cell viabilities were determined by trypan blue dye exclusion, and viable cells were recultured in fresh medium at a density of 3.3×10^5 cells per ml. When cell viabilities dropped to 50%, uninfected cloned T lymphoblasts were added to the cultures. Culture supernatants were stored at -80°C until they were analyzed for RT activity.

RT activity was determined from viral particles precipitated with polyethylene glycol as described by Poesz et al. (21) and Rey et al. (22), with the following modifications. The primer used for these reactions was poly(rA) · poly(dT) (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.), and the mixtures were incubated for 24 h at 37°C . For all experiments, control supernatants harvested from cultures of uninfected T-cell clones were used to determine background levels of RT activity, which were consistently negative. Viral infectivity was also determined by an indirect immunofluorescence antibody test (IFAT), using a modification of the procedure described by Minami et al. (17). Uninfected and FIV-infected T cells were washed in phosphate-buffered saline (PBS) and were fixed for 10 min on ice

in 1.8% formaldehyde in PBS. The cells were washed extensively in PBS, resuspended to a final concentration of 10^7 cells per ml, and spotted onto the wells of microscope slides (Celline Associates, Inc., Newfield, N.J.). The slides were air dried and stored at -80°C . Normal serum obtained from an SPF cat and FIV-specific serum obtained from an FIV-infected cat were diluted 1:50 in PBS and used as primary antibody, and fluorescein-conjugated, affinity-purified anti-feline immunoglobulin G (heavy and light chains) (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) diluted 1:100 was used as a second antibody. In some experiments, a control cat serum specific for FeSFV was also used.

In the first experiment, CD4⁺ T-cell clones 3B4, 3D7, and 3D9 and CD8⁺ T-cell clone 2D9 were infected with the Petaluma isolate of FIV with an RT activity of approximately 5×10^6 cpm/ml. All clones were positive for FIV by IFAT by day 10, which was the earliest time fluorescence was determined, and continued to be FIV positive thereafter (Fig. 2). All clones were negative for FeSFV by IFAT, whereas Crandell feline kidney (CRFK) cells infected with FeSFV were positive when stained with this antiserum but negative when stained with serum from the FIV-infected cat (data not presented). RT activities from culture supernatants from these infected clones are shown in Fig. 3. All supernatants had residual RT activity on day 2 following infection. CD8⁺ clone 2D9 and CD4⁺ clone 3B4 had RT levels above background by day 10, and supernatants from all four clones were positive by day 16. Clone 3D9 had the weakest level of

FIG. 2. Immunofluorescent staining of FIV proteins expressed in feline T-cell clones (experiment 1). CD4⁺ T-cell clones 3D7 (a), 3D9 (b), and 3B4 (c) and CD8⁺ T-cell clone 2D9 (d) were infected with the Petaluma isolate of FIV. Uninfected 3D7 cells are also shown (e).

RT activity and fluorescence (Fig. 2), possibly because this T-cell clone also exhibited the poorest cell growth (data not presented). Clones 3B4 and 3D7 were analyzed by flow cytometry on days 7 and 5, respectively, after infection and were still CD4⁺ CD8⁻, as expected (Fig. 1A). However, because too few cells were available for phenotypic analysis from the cultures of clones 3D9 and 2D9, in vitro infection was repeated with clone 3D7 and two additional CD8⁺ clones, designated 1F6 and 1F7.

In the second experiment, CD4⁺ clone 3D7 and CD8⁺ clones 1F6 and 1F7 were infected with the Petaluma isolate of FIV with approximately 1.24×10^6 cpm of RT activity per ml. Cells from all three clones were positive for FIV when analyzed by IFAT 14 days after infection. RT levels above background were observed in all culture supernatants 7 days after infection (Fig. 4). The highest RT activity of 1.9×10^6

FIG. 3. RT activities in culture supernatants harvested on the indicated days after in vitro infection of feline T-cell clones with the Petaluma isolate of FIV (experiment 1).

FIG. 4. RT activities in culture supernatants harvested on the indicated days after infection of feline T-cell clones with the Petaluma isolate of FIV (experiment 2).

FIG. 5. RT activities in culture supernatants harvested on the indicated days after infection of cloned feline T cells with either the Peeper or Petaluma isolate of FIV (experiment 3).

cpm/ml was found in the supernatant of CD8⁺ clone 1F7 on day 21; however, on day 23 the supernatants from this clone and the CD4⁺ clone 3D7 contained equal RT activities (1.6×10^6 cpm/ml). Analysis of the cell surface phenotypic markers on day 11 after infection showed that the 1F7 cells were still CD8⁺ CD4⁻, although the staining was weak (Fig. 1B).

The Petaluma isolate of FIV has a broad host cell range, and in addition to growing in T cells and macrophages, has been successfully adapted to grow in fibroblastoid cell lines, including CRFK cells (25). The Peeper isolate of FIV, on the other hand, is more restricted in its host cell range, replicating in leukocytes but not in CRFK cells (7). We wished to determine whether the ability of FIV (Petaluma) to infect and replicate in both CD8⁺ and CD4⁺ T cells was a characteristic of this virus, or whether it replicated in both T-cell subsets because it was more promiscuous in its host cell range. To address this question, CD4⁺ 3D7 and CD8⁺ 1F7.G6 T-cell clones were infected with the Peeper isolate of FIV (approximately 3.94×10^6 cpm of RT activity per ml), and as a control, clone 1F7.G6 was also infected with the Petaluma isolate of FIV (approximately 1.24×10^6 cpm of RT activity per ml). In this experiment, cells from all clones infected with either isolate were positive for FIV when analyzed by IFAT on day 13 postinfection. At this time, the percentages of infected cells were calculated by counting total cells and FIV⁺ cells in at least three microscopic fields. Eighty-one percent (62 of 76) of clone 1F7.G6 cells infected with the Peeper isolate were positive, 61% (62 of 101) of clone 1F7.G6 cells infected with the Petaluma isolate were positive, and 58% (70 of 121) of clone 3D7 cells infected with the Peeper isolate of FIV were positive. RT activity was present in all culture supernatants from the beginning of the experiment, although at all time points the RT levels were lower in supernatants from the infected CD8⁺ T-cell clone than in the supernatant from the infected CD4⁺ clone (Fig. 5). The lowest RT levels were found 7 days after infection: 2.17×10^6 cpm/ml in FIV (Peeper)-infected CD4⁺ 3D7 cells, 1.90×10^5 cpm/ml in FIV (Peeper)-infected CD8⁺ 1F7.G6 cells, and 1×10^5 cpm/ml in FIV (Petaluma)-infected CD8⁺ cells. Cell surface phenotypic analysis again showed that the clones retained their original phenotype following infection (Fig. 1). These data demonstrated that different geographical isolates of FIV with different cell tropisms infected and replicated in both CD4⁺ and CD8⁺ feline T-cell subsets and that the majority of cells eventually became infected by either virus in both T-cell subsets.

It appears that both CD4⁺ T and CD8⁺ T cells support replication of the Petaluma strain of FIV. A stock of the

Petaluma isolate of FIV propagated in cultures of CD4⁺ clone 3D7 was used to infect both CD4⁺ and CD8⁺ clones used in this study (experiments 2 and 3), showing that infectivity of CD4⁺ cells does not select for virus with tropism for only CD4⁺ cells. In the first experiment (Fig. 3), RT levels from one CD4⁺ and two CD8⁺ clones were comparable, the fourth CD4⁺ clone yielding the lowest RT activity. In the second experiment (Fig. 4), RT activity was initially highest in the two CD8⁺ clones, although one grew poorly and was terminated on day 16, and by day 23 RT activities in CD8⁺ clone 1F7 and CD4⁺ clone 3D7 were equal. In some cases, low levels of RT activity did appear to correlate with poor growth characteristics of the T-cell clone chosen for infection. For example, clones 3D9 (CD4⁺) and 1F6 (CD8⁺) could not be maintained for more than a few weeks, and the Petaluma virus replicated poorly in these cells (Fig. 3).

In the third experiment (Fig. 5), RT activity was approximately 4- to 11-fold higher in supernatants from the CD4⁺ clone than in the CD8⁺ clone infected with the Peeper isolate of FIV. Furthermore, earlier appearance and higher levels of reverse transcriptase were observed in supernatants of 3D7 T cells infected with the Peeper isolate (maximum of 3.8×10^6 cpm/ml on day 10) than previously observed in supernatants from the same T-cell clone infected with the Petaluma isolate (maximum RT activities of 1.15×10^6 and 1.6×10^6 cpm/ml on days 16 and 23; Fig. 3 and 4). These data suggest that the Peeper isolate may be better adapted than the Petaluma isolate to replicate in CD4⁺ T cells. Infectious molecular clones of the two isolates, in fact, displayed very different *in vitro* host cell preferences; the cloned Peeper isolate replicated in leukocytes but not in CRFK cells, whereas the cloned Petaluma isolate replicated less efficiently in leukocytes than in CRFK cells (20). In two experimental infections of the CD4⁺ 3D7 T-cell clone with either the cloned Peeper or Petaluma virus stock, cells infected with the cloned Peeper virus were strongly positive for FIV by IFAT on days 10, 19, and 23, whereas the cloned Petaluma virus-infected cells were only weakly positive on day 23 (data not shown). Further experiments are needed to enumerate the percentages of FIV-positive cells sequentially following infection of CD4⁺ and CD8⁺ feline T-cell subsets by uncloned and cloned Petaluma and Peeper isolates.

Both the Petaluma and Peeper isolates of FIV induced cytopathic changes in CD4⁺ and CD8⁺ T cells (Table 1). Percentages of viable cells in cultures of uninfected clones 1F7.G6 and 3D7 were compared with percentages of viable cells in cultures of these clones on sequential days after

TABLE 1. Decreased cell viability following infection of CD4⁺ and CD8⁺ T cells with the Petaluma or Peeper isolate of FIV

Day after infection	% of viable cells in culture					
	Expt 2, clone 3D7 (CD4 ⁺)		Expt 3			
	Uninfected	FIV (Petaluma)	Clone 3D7 (CD4 ⁺)		Clone 1F7.G6 (CD8 ⁺)	
			Uninfected	FIV (Peeper)	Uninfected	FIV (Peeper)
2	66	78				
3			91	86	100	91
4	88	87				
5			90	69	85	90
7	67	75	90	66	90	74
9	85	65				
10			86	42 ^a	74	69
11	69	73				
12			83	42	83	52
14	88	43 ^a				
16	87	51				
18	92	53				

^a Uninfected blasts were added to the cultures.

infection with either of the isolates. The Petaluma FIV isolate caused marked cell death in CD8⁺ and CD4⁺ cells between 12 and 14 days after infection. The Peeper isolate was more cytopathic for CD4⁺ cells, inducing a loss in viability to 42% on days 10 and 12 in these cells, compared with 65% on day 12 in CD8⁺ cells.

The precise nature of the cell receptor for FIV is unknown. These studies suggest either that it is not CD4 or that alternative receptor proteins can also be used for virus-cell surface binding. Although the CD4 molecule is the receptor for HIV gp120, individual CD8⁺ CD4⁻ lymphocytes of T-cell lines established from monkeys infected with SIV_{MAC} or from humans infected with HIV replicated the corresponding viruses in vitro (24). However, the authors reported that they were unable to infect CD8⁺ lymphocytes in vitro with SIV_{MAC}, and they hypothesized that infection of immature T cells coexpressing CD4 and CD8 might have occurred in vivo. With our studies on FIV, the use of monoclonal populations of mature T cells expressing only the CD8 determinant as target cells for FIV infection eliminates the possibility that a minor subset of T cells coexpressing CD4 was infected and later modulated the expression of the surface antigen.

Our results raise the possibility that both CD4⁺ and CD8⁺ T lymphocytes harbor FIV in vivo. However, in two studies, only CD4⁺ lymphocytes were depleted in cats experimentally infected with FIV (Petaluma). Ackley and coworkers (2) reported a significant and progressive decline with time of circulating CD4⁺ T cells and an increase in CD8⁺ lymphocytes in infected cats compared with noninfected littermates. Barlough and associates (3) also documented a similar time-related in vivo depletion of CD4⁺ lymphocytes in FIV-infected cats, with no significant changes in the CD8⁺ lymphocyte population. This apparent dichotomy in the in vitro and in vivo behavior of FIV indicates that in vivo events which lead to selective depletion of the CD4⁺ subset are complex and cannot be explained merely by cell tropism and viral cytopathicity. Additional studies are needed to determine the capacity for FIV to replicate in T-cell subsets in vivo.

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