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Induction of Feline Immunodeficiency Virus-Specific Cytolytic T-Cell Responses from Experimentally Infected Cats

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We have examined the in vitro induction and activity of feline immunodeficiency virus (FIV)-specific cytolytic T cells obtained from cats experimentally infected for 7 to 17 weeks or 20 to 22 months with the Petaluma isolate of FIV. Normal or FIV-infected autologous and allogeneic T lymphoblastoid cells were used as target cells in chromium-51 or indium-111 release assays. When effector cells consisted of either fresh peripheral blood mononuclear cells or concanavalin A- and interleukin-2-stimulated cells, only low levels of cytotoxicity were observed. However, the levels of FIV-specific cytotoxicity were consistently higher in both groups of cats following in vitro stimulation of the effector cells with irradiated, FIV-infected autologous T lymphoblastoid cells and interleukin-2. The effector cells lysed autologous but not allogeneic FIV-infected target cells and were composed predominantly of $CD8^+$ T cells, indicating that the FIV-specific cytotoxicity measured in this system is mediated by $CD8^+$, major histocompatibility complex class I-restricted T cells. These studies show that FIV-specific cytolytic T cells can be detected as early as 7 to 9 weeks postinfection, and they define a system to identify virus-encoded epitopes important in the induction of protective immunity against lentiviruses.

Feline immunodeficiency virus (FIV) is currently considered a valuable small-animal model for human immunodeficiency virus (HIV)-induced AIDS in humans (2, 4, 8, 11, 17, 24, 33, 39, 43, 53–55). The observation that humans may be infected with HIV-1 for long periods without clinical illness suggests that a natural immune mechanism must be capable of inhibiting the spread of viral infection (49). Cell-mediated immune mechanisms, especially HIV-1-specific cellular cytotoxicity, may be particularly important in host defense against this virus by destroying virus-infected cells (12, 16, 25, 34, 35, 46) and suppressing viral replication (18, 44, 50). Cytotoxic T lymphocytes (CTL) directed against HIV-1 have been detected in HIV-infected humans (9, 10, 14, 16, 20, 22, 23, 30, 35, 36, 38, 46–48), in human volunteers vaccinated with soluble gp160 (31), and in chimpanzees immunized with recombinant vaccinia virus expressing HIV-1 envelope glycoproteins (56). Simian immunodeficiency virus (SIV)-specific CTL were also observed in infected (26–28, 45, 52) and vaccinated (13, 37) primates. Although most studies reported that the effector cells were $CD8^+$ major histocompatibility complex (MHC) class I-restricted T cells, HIV-specific $CD4^+$ MHC class II-restricted CTL were observed in HIV-seronegative humans (40), vaccinated human volunteers (31), and chimpanzees (56). Similarly, SIV *gag*-specific $CD4^+$ MHC class II-restricted CTL were detected in immunized macaques (29).

Compared with SIV and HIV infections, little is known about cell-mediated immune responses in FIV-infected cats. Research in our laboratory is directed toward elucidating the FIV-specific cellular immune responses that participate in the development of protective immunity or immunopathology associated with lentiviral infection or vaccination. Here we report, for the first time, the detection of MHC-restricted, FIV-specific cytotoxic T lymphocyte responses in cats experimentally infected with FIV.

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MATERIALS AND METHODS

Experimental animals. The 13 domestic, specific-pathogen-free (SPF) cats (Liberty Laboratories, Liberty Corner, N.J.) selected for this study were serologically negative for FIV, feline syncytium-forming virus, feline coronavirus, and feline leukemia virus. Cats were housed under SPF conditions at the Laboratory of Animal Research and Resources Facilities, Texas A&M University, College Station. All animals were maintained in accordance with the Guide for the Care and the Use of Laboratory Animals, Department of Health and Human Services.

Viruses. The Petaluma isolate of FIV used for in vitro infection of target cells was obtained from the National Institute of Allergy and Infectious Diseases AIDS Research and Reference Reagent Program (catalog no. 236) and was prepared for use as a virus stock as described by Brown et al. (5). Heparinized FIV-infected donor blood used to inoculate the SPF cats was collected from a cat with experimentally induced FIV Petaluma infection and was kindly provided by Niels C. Pedersen, University of California, Davis.

Experimental infection. Two separate groups of cats were inoculated intraperitoneally with heparinized FIV-infected blood. In the first group (acutely infected), cats QB2, QB3, and QB5 were inoculated intraperitoneally at 6 months of age with 1.0 ml of FIV-infected blood and were monitored from 1 to 17 weeks postinfection. Cats QA2 and QB4 served as uninfected controls for this group. Virus-specific cellular immune responses were examined weekly for the first month and biweekly for the second month after FIV inoculation. In the second group (chronically infected), cats EK3, ET4, and EZ3 were inoculated at 1 year of age with FIV (57) and were monitored from 59 to 89 weeks postinfection. Cats EC4, ED3, EK6, ET5, and EY5 served as uninfected controls for this group.

Serologic determinations. Serum antibody to FIV was monitored by enzyme-linked immunosorbent assay (IDEXX, Portland, Maine) and by Western immunoblot analyses with virus from infected Crandell feline kidney cells, goat anti-cat immunoglobulin G conjugated with alkaline phosphatase as the secondary antibody, and 5-bromo-4

chloro-3-indolylphosphate toluidinium–Nitro Blue Totrazolium (BCIP-NBT) substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.).

Flow cytometry. The percentages of $CD4^+$ and $CD8^+$ T cells in peripheral blood mononuclear cell (PBMC) or in lymphoblastoid cell lines were determined as described previously (5) by indirect immunofluorescence staining and flow cytometry with mouse monoclonal antibodies (kindly provided by Chris Ackley and Max Cooper, University of Alabama, Birmingham) specific for feline CD4 (1) and CD8 (19) determinants.

PCR analysis. PBMC were obtained from heparinized blood of control and FIV-infected cats by Ficoll-sodium diatrizoate (Histopaque-1077; Sigma Chemical Co., St. Louis, Mo.) density gradient centrifugation. Genomic DNA was extracted from the cells by the technique of Weintrub et al. (51). An aliquot of 5×10^5 cells was washed in phosphate-buffered saline and pelleted at $250 \times g$ for 5 min. The resulting pellet was resuspended in 25 μl of solution A (100 mM KCl, 10 mM Tris [pH 8.3], 2 mM $MgCl_2$)–25 μl of solution B (10 mM Tris [pH 8.3], 2 mM $MgCl_2$, 1% Tween 20, 1% Nonidet P-40, 120 μg of proteinase K per ml). The cell lysate was incubated at 60°C for 1 h and then subjected to heat inactivation of proteinase K in a boiling-water bath for 10 min. No further purification of the sample was necessary. Polymerase chain reaction (PCR) primers (upstream [5′-TACATCTGGTGACTCTTCGC-3′] and downstream [5′-CTTTCCTCCTCCTACTCCAA-3′]) for the *gag* gene were chosen from the published sequence of the FIV genome (42) by using the OligoTM software (MBI, Plymouth, Minn.) and were synthesized by Biosynthesis, Denton, Tex. The PCRs were carried out with 100-μl reaction volumes (0.25 mM nucleotide mix, 5 U of *Taq* DNA polymerase [Promega, Madison, Wis.], 0.15 μM upstream primer, 0.15 μM downstream primer, PCR reaction buffer containing 1.5 mM $MgCl_2$ [Promega]) for 30 or 40 cycles, with a denaturation temperature of 94°C for 1 min, an annealing temperature of 52°C for 30 s, and an extension temperature of 72°C for 1 min. Ten percent of the resulting PCR sample was run on a 1% agarose gel at 60 V for 2 h and transferred by Southern blotting to nylon membranes (GeneScreen Plus; Dupont NEN, Boston, Mass.). The cloned *gag* long terminal repeat DNA (kindly provided by John Elder, Scripps Research Institute, La Jolla, Calif.) was labeled by the random-primer method with [^{32}P]dCTP (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The membrane was hybridized overnight with the *gag* probe (2×10^9 cpm) by using the rapid-hybridization solution from Amersham, Arlington Heights, Ill., as specified by the manufacturer. The blot was washed twice in 2× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7])–0.1% sodium dodecyl sulfate (SDS) for 10 min at room temperature, once in 1× SSPE–0.1% SDS for 15 min at 65°C, twice in 0.5× SSPE–0.1% SDS for 10 min at 65°C, once in 0.2× SSPE–0.1% SDS for 30 min at 65°C, and once in 0.1× SSPE–0.1% SDS for 30 min at 65°C. The membrane was exposed to autoradiography film (Amersham Hyperfilm MP) at room temperature overnight.

Preparation of target cells for cytotoxicity assays. Heparinized blood was drawn from the cats before FIV infection. PBMC were isolated by Ficoll-sodium diatrizoate density gradient centrifugation and were used to prepare T lymphoblastoid cells. The PBMC were stimulated with 5 μg of concanavalin A (ConA) (Sigma Chemical Co.) per ml of complete RPMI 1640 medium (5) for 3 days at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells were cultured at a concentration of 1.5×10^6 to 3×10^6/ml of complete medium in a 24-well plate (Costar, Cambridge, Mass.). The ConA-stimulated PBMC were then resuspended in complete medium supplemented with 10% bovine T-cell growth factor (TCGF), prepared as described previously (6), and 20 U of human recombinant interleukin-2 (IL-2; Boehringer Mannheim Biochemicals) per ml and were cultured for an additional 1 to 2 weeks (5). The cells were aliquoted and stored in liquid nitrogen for later use as normal autologous target cells. The lymphoblastoid cells were infected in vitro with FIV Petaluma as described previously (5). A stock of virus grown in feline T cells with a reverse transcriptase (RT) activity of 1.24×10^6 cpm/ml was used for in vitro infection. Uninfected and infected cells were maintained in 24-well plates at a density of 3.3×10^5 cells per ml of complete medium supplemented with TCGF and IL-2. Cell viabilities were monitored by trypan blue dye exclusion. When the percentage of viable cells decreased to 70% or less, an equal number of uninfected autologous lymphoblasts were added to the cultures. The viral infectivity of cultured cells was determined by measuring RT activity in culture supernatants (5) and by the indirect immunofluorescent-antibody test (IFAT) with normal, feline syncytium-forming virus-specific, and FIV-specific feline sera as described previously (5). The FIV-infected cells were aliquoted and stored in liquid nitrogen for use as autologous or allogeneic target cells and as stimulator cells for the establishment of FIV-specific T-cell lines.

Cytotoxic effector cells. Three kinds of effector cells were used in this study: fresh PBMC, ConA- and IL-2-stimulated PBMC, and FIV-stimulated T-cell lines. PBMC were obtained from control cats or from infected cats at various times following experimental FIV infection. To prepare ConA blasts, PBMC were stimulated with 5 μg of ConA per ml for 3 days and then with 10% bovine TCGF and 20 U of IL-2 per ml for an additional 4 to 5 days. FIV-specific T-cell lines were established by repeated stimulation of T cells with FIV. Briefly, 2×10^6 to 3×10^6 PBMC were cultured for 1 week with 1.5×10^5 to 2×10^5 irradiated (10,000 rads from a ^{60}Co source) autologous FIV-infected lymphoblastoid cells in complete medium in 1.5-ml cultures in 24-well plates. Viable responder cells were purified by Ficoll-sodium diatrizoate density gradient centrifugation, and 10^6 cells were restimulated at weekly intervals with irradiated, autologous FIV-infected PBMC plus 20 U of IL-2 per ml of complete medium. The cells were subcultured 3 to 4 days after antigenic stimulation. At 4 to 7 days after antigenic stimulation, viable cells were purified by Ficoll-sodium diatrizoate density gradient centrifugation and used as effector cells in the cytotoxicity assay. Cells cultured with irradiated, autologous FIV-infected cells for 1.5 to 4 weeks were assayed. When enough cells were available at the time of the cytotoxicity assay, the percentages of $CD4^+$ and $CD8^+$ T cells in the effector cell population were determined by indirect immunofluorescence and flow cytometry (5).

Cytotoxicity assay. Chromium-51 (3, 46) and indium-111 (7) release assays were compared for efficiency of labeling feline target cells. For the ^{51}Cr release assay, 10^6 target cells were pelleted by centrifugation and labeled with 50 μCi of $Na^{51}CrO_4$ (NEZ-030S; Du Pont NEN) for 1 to 3 h at 37°C with gentle agitation at 30-min intervals and then washed three times with complete medium. For the indium-111 release assay, 10^6 target cells were labeled with 5 μCi of indium-111 oxine (Medi-Physics, Inc., Arlington Heights, Ill.) for 15 min at 37°C and then washed five times with complete medium. A fixed number of ^{51}Cr-labeled (5×10^4) or ^{111}In-labeled (5 ×

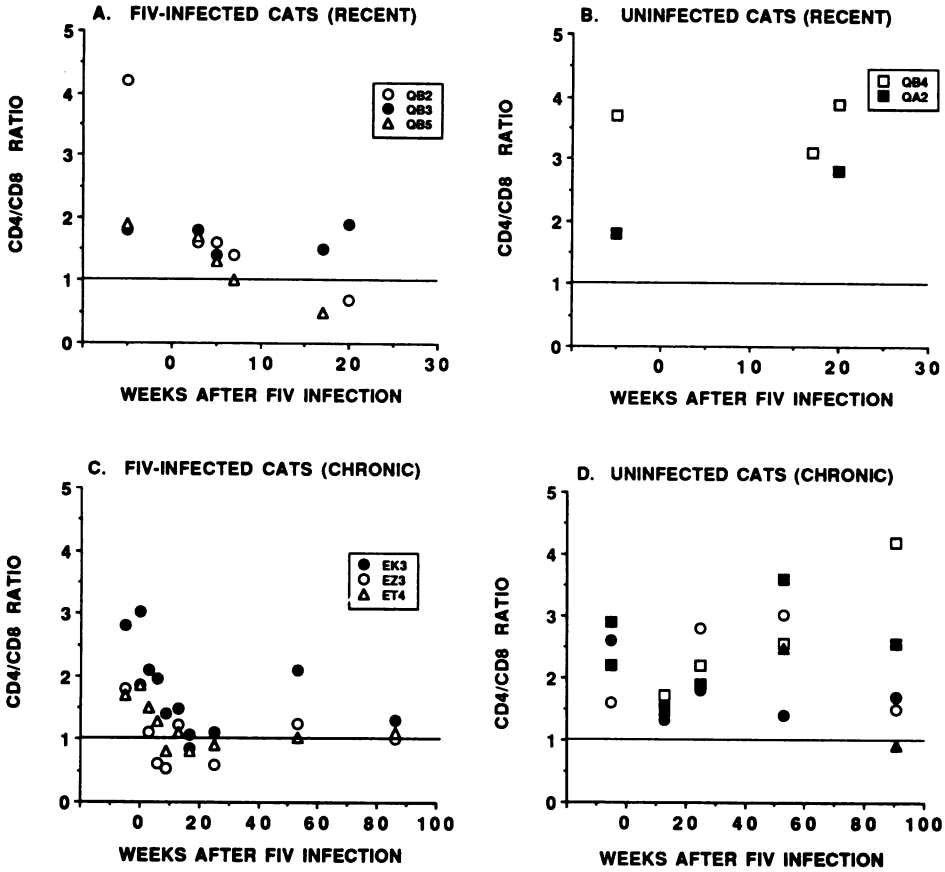

FIG. 1. $CD4^+/CD8^+$ T-cell ratio in PBMC from FIV-infected cats. (A) Cats acutely infected with FIV (group 1); (B) control SPF cats for group 1; (C) cats chronically infected with FIV (group 2); (D) control SPF cats for group 2.

10^3) target cells was added to each well of 96-well V-bottomed plates (Costar). Effector cells were added in triplicate at various effector/target (E/T) cell ratios in a total volume of 200 μl per well. When fresh PBMC and ConA blasts were used as effector cells, E/T ratios of 100:1, 50:1, 25:1, and 12.5:1 were used. When FIV-specific T-cell lines were used as effector cells, E/T ratios of 20:1, 10:1, 5:1, and 2.5:1 were used. The plates were centrifuged at 250 × *g* for 1 to 2 min, incubated at 37°C for 4 h, and then centrifuged again at 250 × *g* for 5 min. Supernatants (100 μl) were collected from each sample and counted in a gamma counter. The maximum isotope release was determined by using triplicate samples of radiolabeled target cells subjected to three cycles of freezing and thawing, followed by centrifugation at 5,000 × *g* for 5 min. Spontaneous release of the isotope was determined by using supernatants of radiolabeled target cells incubated in the assay plate in the absence of effector cells. Spontaneous isotope release was less than 15% of the maximum isotope release for all target cells used in the assay. The percentage of specific cytotoxicity or isotope release was calculated as 100 × (mean cpm test release − mean cpm spontaneous release)/(mean cpm maximum release − mean cpm spontaneous release) for triplicate samples.

RESULTS

Experimental infection of cats with FIV. The presence of FIV-specific antibody in infected cats was determined weekly following FIV inoculation of acutely infected cats QB2, QB3, and QB5. The cats seroconverted by 5, 7, and 4 weeks postinfection, respectively. The ratios of $CD4^+$ to $CD8^+$ T cells in PBMC declined in two of the cats, QB2 and QB5, dropping to <1.0 by 20 weeks postinfection (Fig. 1A). In contrast, during the same period, the ratio of $CD4^+$ to $CD8^+$ T cells remained >1 in FIV-infected cat QB3 (Fig. 1A) and in control cats QA2 and QB4 (Fig. 1B). Cats EK3, ET4, and EZ3, which were chronically infected with FIV, seroconverted by 8 weeks postinfection (57). The ratios of $CD4^+$ to $CD8^+$ T cells in the PBMC of these cats dropped to 1 or <1 by 25 weeks postinfection and remained low (through 86 weeks postinfection) (Fig. 1C). In contrast, control cats for the chronically infected group did not experience a comparable decline in the $CD4^+$ to $CD8^+$ T-cell ratios (Fig. 1D). Serum samples from all FIV-infected cats reacted with FIV on Western blots (data not shown). The presence of virus in both acutely and chronically infected cats was confirmed by PCR analysis of PBMC obtained at the time of this study (Fig. 2). *gag* sequences, which were absent from PBMC of all control cats (lanes 3, 6, 8, and 10) could be demonstrated in PBMC of all acutely (lanes 2, 5, and 7) and chronically (lanes 9, 11, and 13) infected cats.

In vitro infection of ConA blasts with FIV. ConA- and IL-2-stimulated lymphoblastoid cell lines were established from cats QB2, QB3, QB5, and EK3 before the cats were experimentally infected with FIV. The cells were maintained in culture for 1 to 3 weeks and were then infected with FIV Petaluma. Uninfected cell lines could be maintained for up to 7 weeks with cell viabilities of 85 to 95%. However, by 6 to

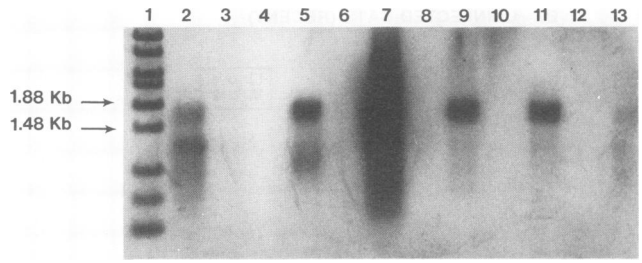

FIG. 2. Detection of FIV *gag* sequences in PBMC of experimentally FIV-infected cats by PCR. PBMC from acutely infected cats QB2, QB3, and QB5 are shown in lanes 2, 5, and 7, respectively. PBMC from chronically infected cats EK3, ET4, and EZ3 are shown in lanes 9, 11, and 13, respectively. PBMC from uninfected cats QA2, QB4, EC4, and EK6 are shown in lanes 3, 6, 8, and 10, respectively. Molecular size markers are shown in lane 1. All lanes represent PCR assays of 40 cycles, except the assays with PBMC from cats QB3, EK3, and ET4, for which 30 cycles were used.

8 days after in vitro infection with FIV, cell viabilities dropped to 60 to 75%, and addition of autologous, uninfected lymphoblastoid cells was required to maintain the cell lines. Cells were examined by IFAT for the presence of viral antigen, and by 6 to 8 days after in vitro infection, 60 to 80% of the cells expressed FIV (Fig. 3C). Normal lymphoblastoid cells were negative by IFAT for FIV (Fig. 3A), and FIV-infected cells did not stain with normal feline serum (Fig. 3B) or with an antiserum directed against feline syncytium-forming virus (Fig. 3D). Viral replication was confirmed by measuring RT activity in the culture supernatants of FIV-infected cells collected over a 3-week period (Fig. 4). Peak levels of RT were observed in supernatants on day 8 postinfection, coincident with the high percentage of FIV^+ cells visualized by IFAT. Cell surface phenotypic analyses of FIV-infected cells showed that most of the cells expressed CD4 (data not shown).

Uninfected and FIV-infected T-cell lines were similarly established from PBMC of chronically infected cats ET4 and EZ3 approximately 1 year after infection. Although cell lines were established from these two cats, which had a latent FIV infection, none of the uninfected cell lines expressed viral antigen during the culture period as determined by IFAT (data not shown).

Target cell labeling. ^{51}Cr and ^{111}In were compared for their efficiency of labeling of both uninfected and FIV-infected feline T cells (Table 1). Maximum labeling of target cells with ^{51}Cr occurred when cells were incubated with the isotope for 3 h. Because the spontaneous release of the isotope was 15% or less, these conditions were used in ^{51}Cr release assays. However, equally efficient incorporation of radioactivity was achieved with 10-fold fewer target cells labeled with

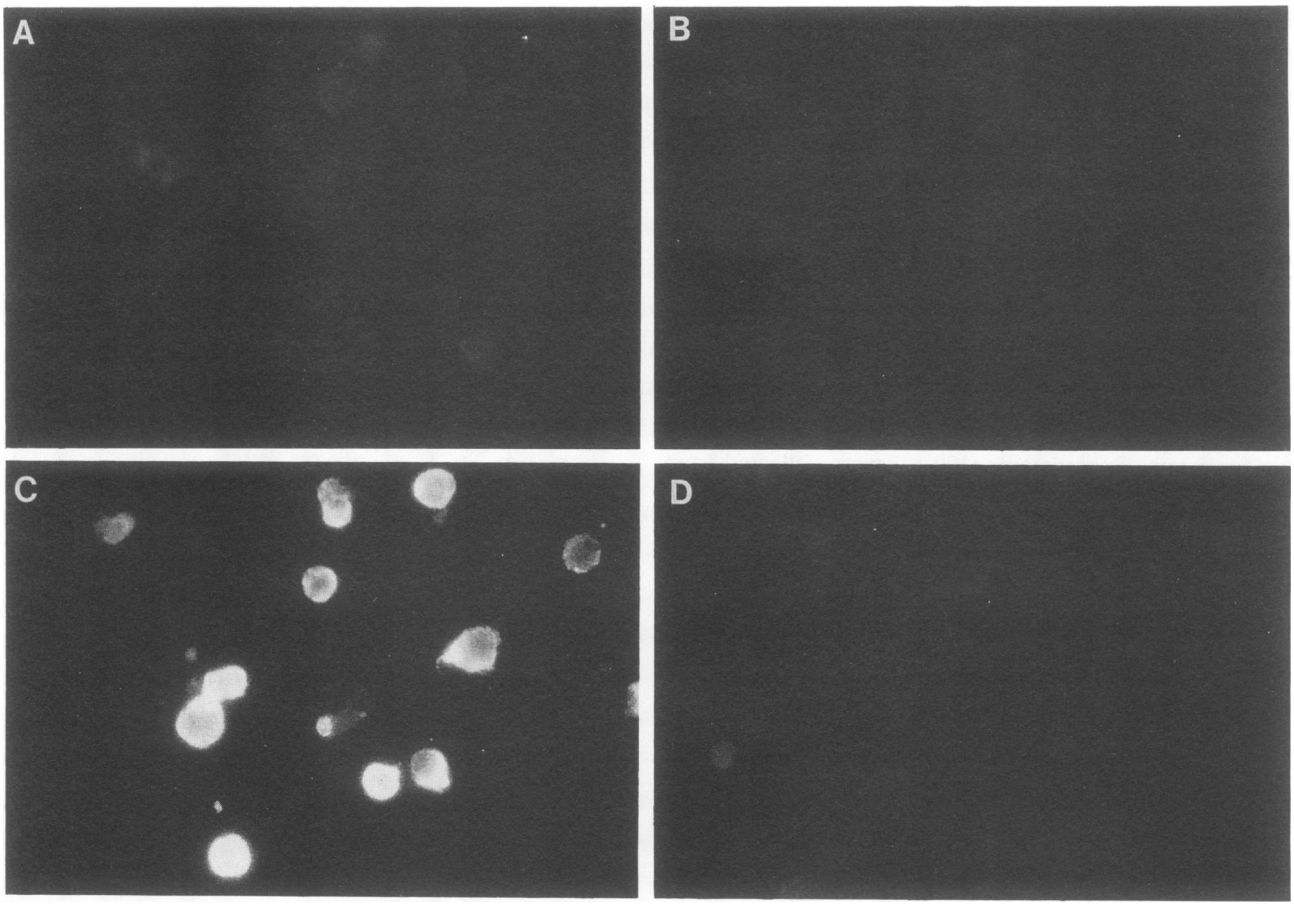

FIG. 3. Immunofluorescence staining of FIV proteins expressed in feline lymphoblastoid cells obtained from cat QB2 8 days after in vitro infection. Uninfected cells (A) and FIV-infected cells (B to D) were stained with normal feline serum (panel B), FIV-specific feline serum (panels A and C), or feline syncytium-forming virus-specific feline serum (panel D).

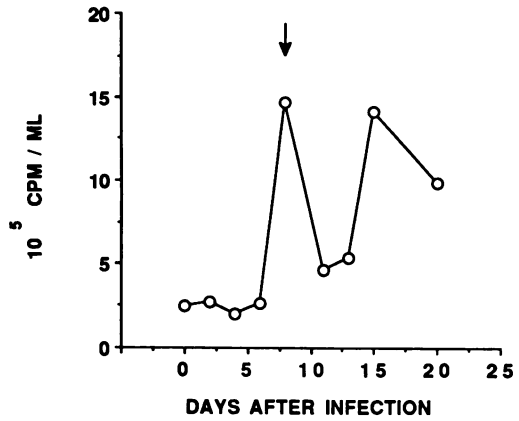

FIG. 4. RT activities in culture supernatants harvested on the indicated days after in vitro infection of feline lymphoblastoid cells obtained from cat QB2. The arrow indicates that autologous uninfected cells were added to the culture 8 days after infection and on alternate days thereafter.

^{111}In for 15 min, with comparable levels of spontaneous isotope release. Because cytotoxicity assays with ^{111}In-labeled target cells required 10-fold fewer target cells and effector cells, this isotope was selected for use in most of the experiments.

Detection of FIV-specific cytotoxic responses in FIV-infected cats. FIV-specific cytotoxic T-cell activity was examined in fresh PBMC, ConA blasts, and T-cell lines stimulated for 1.5 to 4 weeks with autologous, irradiated FIV-infected lymphocytes. No cytotoxicity of any target cell was detected with PBMC obtained from any of the acutely infected cats QB2 (Table 2), QB3 (Table 2; Fig. 5A), or QB5 before or up to 4 weeks following FIV infection. At 7 weeks postinfection, FIV-specific cytotoxicity ranging from 3 to 7% (with an E/T ratio of 100:1) was observed with ConA- and IL-2-stimulated lymphoblasts obtained from all three FIV-infected cats. An example of cytotoxicity mediated by lymphoblasts obtained from cat QB3 is shown in Fig. 5B. FIV-specific T-cell lines established from PBMC obtained 9 weeks postinfection exhibited specific killing of autologous FIV-infected target cells of 7.7% (QB2), 15.8% (QB3; Fig. 5C), and 28.9% (QB5) at an E/T ratio of 20:1. Similar levels of FIV-specific cytotoxicity from these three cats were again observed in cell lines established 17 weeks postinfection (Fig. 5D; Table 2). No killing of uninfected autologous or FIV-infected mismatched target cells occurred.

TABLE 1. Comparison of the efficiency of radiolabeling feline T-cell blasts with ^{51}Cr and ^{111}In

Isotope and labeling period[a]	Maximum release of radioactivity incorporated by feline T cells[b]	
	Uninfected	FIV infected
^{51}Cr		
1.0 h	5,240 ± 128 (3.8)	2,214 ± 85 (5.8)
1.5 h	6,164 ± 272 (4.3)	3,601 ± 105 (6.4)
3.0 h	15,439 ± 340 (5.2)	7,969 ± 279 (9.2)
^{111}In		
15 min	10,688 ± 260 (2.3)	12,669 ± 157 (10.6)

[a] Cells were labeled with either ^{51}Cr (5×10^4 cells per well) or ^{111}In (5×10^3 cells per well) for the times indicated.

[b] Data are shown as the mean cpm ± standard deviation of triplicate samples. Numbers in parentheses indicate the spontaneous release calculated as a percentage of the maximum release.

TABLE 2. Cytotoxicity responses and phenotypic analyses of feline T cells stimulated in vitro with FIV-infected cells

Cat	Days in culture	% Specific cytotoxicity[a]	CD8/CD4 ratio of effector cells[b]
Normal			
QA2	12	0.6	1.8
QB4	12	0.4	1.8
FIV infected			
QB2	0[c]	0	0.4
	16	6.4	3.9
QB3	0[c]	0	0.5
	16	17.5	10.8
EK3	0[c]	1.6	0.8
	24	30.5	10.0
ET4	0[c]	0	0.9
	14[d]	11.7	4.7
	19[d]	8.7	32.7
EZ3	0[c]	0	1.0
	19	14.6	3.6

[a] Data obtained by using E/T ratios of 100:1 for PBMC and 20:1 for FIV-stimulated T-cell lines in a 4-h ^{111}In release assay.

[b] The ratios of $CD8^+$ to $CD4^+$ T cells in the effector cell populations were determined at the time of assay.

[c] Freshly isolated PBMC.

[d] Data from two separate T-cell lines are presented.

At 22 weeks postinfection, PBMC from control cats QA2 and QB4 were stimulated for 12 days with autologous, FIV-infected lymphocytes and were tested for cytotoxicity. There was no detectable killing of uninfected autologous target cells, FIV-infected autologous target cells, or FIV-infected allogeneic target cells (Table 2).

PBMC from the chronically infected cats were then examined for FIV-specific cytotoxicity. PBMC obtained 59 weeks postinfection from cat EK3 exhibited low levels of killing against FIV-infected autologous target cells, reaching a maximum of about 10% with an E/T ratio of 50:1 (Fig. 6A). ConA- and IL-2-stimulated lymphoblasts derived from EK3 PBMC obtained at 61 weeks postinfection exhibited a similar level of killing at all E/T ratios tested (Fig. 6B). On other occasions, no cytotoxicity could be detected with either PBMC or lymphoblasts from this cat (data not shown). FIV-specific T-cell lines established from this cat 62 to 72 weeks postinfection showed a higher specific lysis of FIV-infected autologous target cells. For example, cytotoxicity ranging from 19.0 to 30.5% with an E/T ratio of 20:1 in a cell line stimulated for 10 or 24 days is shown in Fig. 6C and D, respectively. No killing of FIV-infected allogeneic target cells was seen, but low levels of killing of uninfected autologous target cells occasionally occurred (Fig. 6C).

No cytotoxicity was observed with either PBMC or ConA blasts from infected cats ET4 and EZ3 when tested 82 to 89 weeks postinfection (data not shown). However, FIV-specific T-cell lines established from PBMC of these two cats at 82 to 84 weeks postinfection exhibited 11.7 and 14.6% specific killing of autologous FIV-infected target cells, with an E/T ratio of 20:1 (Table 2). Background levels of cytotoxicity against uninfected autologous cells or FIV-infected allogenic targets were less than 4%.

Cell surface phenotype analysis of the effector CTL population. Stimulation of PBMC from infected cats with FIV-infected T cells and IL-2 appeared to selectively induce the proliferation of $CD8^+$ T cells, since cells cultured with antigen for 14 to 24 days contained a higher proportion of

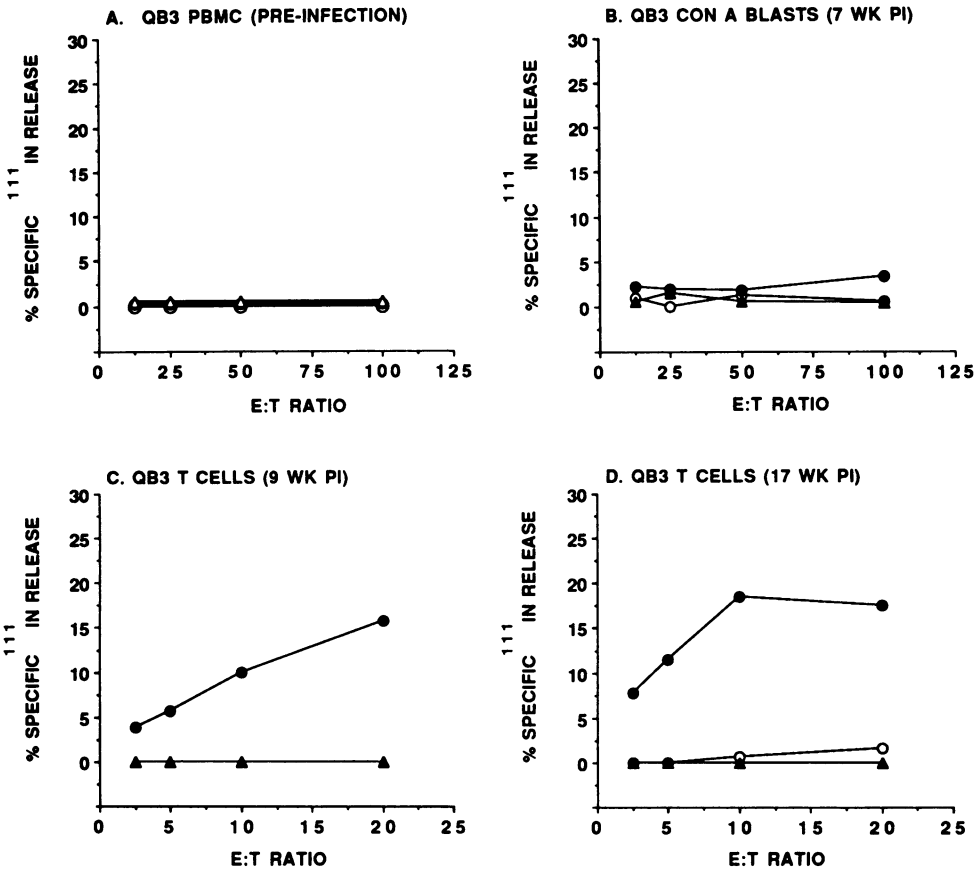

FIG. 5. FIV-specific cytotoxicity in acutely infected cat QB3. PBMC obtained before infection (A), lymphoblastoid cells obtained 7 weeks postinfection (PI) (B), an FIV-specific T-cell line established from PBMC obtained 9 weeks postinfection and cultured for 21 days with irradiated autologous FIV-infected T cells (C), and an FIV-specific T-cell line established from PBMC obtained 17 weeks postinfection and cultured for 16 days with irradiated autologous FIV-infected T cells (D) were used as effector cells. Normal autologous cells (○), FIV-infected autologous cells (●), and FIV-infected allogeneic cells (▲) were used as target cells.

$CD8^+$ T cells than unstimulated PBMC did (Table 2). Furthermore, the degree of FIV-specific cytotoxicity tended to correlate with the proportion of $CD8^+$ T cells in the effector cell population. Low levels of killing of normal autologous target cells were occasionally observed (Fig. 6), whereas similar levels of cytotoxicity directed against allogeneic FIV-infected target cells were never detected (Fig. 5 and 6). Although the MHC class I phenotypes for these cats have not been determined, our results are consistent with the hypothesis that lysis of FIV-infected target cells is mediated in part by MHC class I-restricted, $CD8^+$ CTL.

DISCUSSION

The results of this study demonstrate the presence of FIV-specific, MHC-restricted CTL precursor cells in the peripheral blood of cats experimentally infected with FIV. Two groups of cats were included in the study: acutely infected (for less than 5 months) and chronically infected (for 1 to 1.5 years) cats. For the duration of this study, FIV-infected cats remained clinically healthy, although a decrease in the ratio of $CD4^+$ to $CD8^+$ T cells in PBMC by 20 to 25 weeks postinfection in five of the six cats and the early appearance of a humoral immune response in all animals were observed as previously reported for FIV-infected cats (2, 4, 39, 43). Furthermore, at the time of this study, the FIV genome was detected by PCR analysis in PBMC of all acutely and chronically infected cats. As with humans infected with HIV, cats infected with FIV may remain clinically healthy for years, suggesting that FIV-specific host immunity may play a role in preventing the development of AIDS-like disease in these animals.

To examine the potential role of FIV-specific cytotoxic T cells in the protective immune response against FIV in infected cats, we adopted a modified cytotoxicity protocol in which ^{111}In was used to radiolabel lymphoblastoid target cells (7). The use of this isotope enabled the detection of cytotoxicity with 10-fold fewer target and effector cells, an important consideration when working with an outbred population of small animals. FIV-specific cytotoxicity was first detected in ConA- and IL-2-activated T cells from all three acutely infected cats 7 weeks after infection, but detection of CTL activity in either PBMC or lymphoblasts in the chronically infected cats was inconsistent. However, FIV-specific cytotoxic responses in all cats were considerably augmented following in vitro antigenic stimulation of T cells. This finding is explained most easily by a clonal expansion and differentiation of CTL precursor populations in PBMC into mature CTL, as has been demonstrated with lymphocytes from HIV-seropositive humans and SIV-infected primates (9, 10, 16, 21, 23, 38).

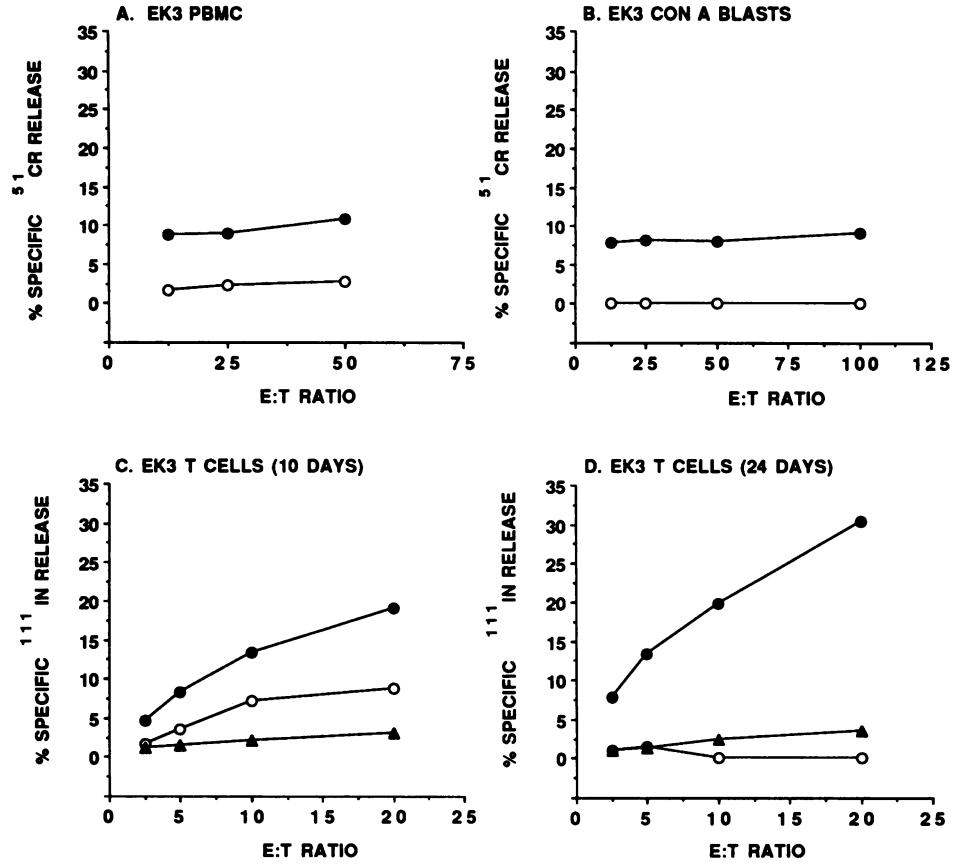

FIG. 6. FIV-specific cytotoxicity in chronically infected cat EK3. PBMC obtained 59 weeks postinfection (A), lymphoblastoid cells obtained 61 weeks postinfection (B), and an FIV-specific T-cell line established from PBMC obtained 72 weeks postinfection and stimulated in vitro with FIV-infected autologous T cells for 10 (C) or 24 (D) days were used as effector cells. Symbols are the same as those defined for Fig. 5.

The intensities of FIV-specific cytotoxic responses by PBMC with or without a brief stimulation with the T-cell mitogen ConA and IL-2 were lower than those reported for PBMC or ConA blasts of HIV-seropositive humans (14, 19, 22, 30, 46, 48) or SIV-infected primates (26–28). In addition, we were unable to detect cytotoxic T-cell responses in lymphoblasts sampled before 7 weeks postinfection, whereas SIV-specific cytotoxicity mediated by PBMC was detected as early as 2 weeks after infection of macaques (26). Several studies have reported the induction of HIV envelope-specific CTL from PBMC of seronegative individuals following a brief coculture with autologous HIV-infected lymphoblasts (9, 16, 23) or after stimulation with gp120-pulsed monocytes (40), indicating the existence of HIV envelope-reactive precursor cells in uninfected individuals. In contrast, we were unable to induce FIV-specific CTL by in vitro stimulation of PBMC from two uninfected cats with autologous FIV-infected T cells. Therefore the higher levels of lentivirus-specific CTL activity in humans and simians than in cats may be due to a higher frequency of lentivirus-reactive CTL precursors in humans and nonhuman primates. Alternatively, the FIV-specific CTL may be directed against antigens other than envelope proteins, since *gag*-specific CTL precursor populations have not been detected in HIV-seronegative individuals (9, 21, 22).

Another explanation for the comparatively low levels of specific cytotoxicity observed in our studies may be the use of FIV-infected, IL-2-activated T-cell blasts as target cells. To maintain the cells in a viable state, we added an equivalent number of uninfected autologous lymphoblasts to the infected-cell cultures at frequent intervals so that the percentage of infected cells in the target cell population at any given time was in the range of 60 to 80%. Despite this limitation, infected T cells are the natural target cells in an infected animal, and therefore the cytotoxicity measured in vitro was probably directed against viral antigens which would be encountered during a natural infection in vivo. The target cells consisted of a mixture of $CD4^+$ and $CD8^+$ T cells, and although we have shown that both T-cell subsets can be infected by FIV in vitro (5), we have not yet determined the relative susceptibilities of the two cell types to CTL-mediated killing.

The frequency and activity of HIV- and SIV-specific CTL appear to decline with the progression of disease (15, 16, 27, 32, 45, 46). By using FIV-stimulated T-cell lines, we found that FIV-specific killing was mediated by lymphocytes sampled from all three acutely infected cats at 9 and 17 weeks postinfection and that even higher levels of cytotoxicity were effected by T cells obtained from all three chronically infected cats approximately 1.5 years postinfection. This finding is probably explained by the clinically healthy status of the chronically infected cats. Even though the $CD4^+$/$CD8^+$ T-cell ratios in PBMC had dropped significantly, the ratios were still approximately 1.0. The demonstration of

CTL precursors in clinically healthy FIV-infected cats up to 1.5 years after the initial exposure to the virus supports the current hypothesis that cytotoxic T lymphocytes may play an important role in host defense against lentiviral infections (12, 16, 25, 34, 41, 46).

Cell surface phenotypic analysis of several of the CTL lines analyzed in this study indicated that most effector cells in the population expressed CD8 and are therefore likely to be MHC class I restricted. This is not surprising since autologous FIV-infected T cells were used as antigen for stimulating the cell lines in the absence of exogenous antigen-presenting cells. Although we have no information regarding the MHC class I or class II phenotypes of our target cells, virus-specific killing was apparently MHC restricted, since allogeneic infected T cells were never lysed by CTL. For this reason, cytotoxicity mediated by feline lymphocytes was not due to natural killer-like killing, although we cannot rule out the possibility that $CD4^+$ MHC class II-restricted T cells also contribute to cytotoxicity. The use of cloned T-cell lines would more precisely define the effector cells mediating FIV-specific killing of infected target cells.

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