Studies on feline CD8$^+$ T cell non-cytolytic anti-feline immunodeficiency virus (FIV) activity

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Summary. CD8$^+$ T cells in FIV-infected cats inhibit feline immunodeficiency virus (FIV) replication by producing a soluble factor(s). In the present study, four SPF cats were experimentally infected with FIV. The period during which the anti-FIV activity of CD8$^+$ T cells became detectable was investigated, and the presence or absence of this activity in the lymph nodes and spleen was examined. Furthermore, we investigated which step(s) of the FIV replication cycle are affected by this antiviral activity. This antiviral activity became detectable five weeks after FIV infection in early cases, and it was simultaneous with or one week after the induction of humoral immunity. All cats having CD8$^+$ T cells with anti-FIV activity in the peripheral blood also possessed CD8$^+$ T cells with anti-FIV activity in the lymph nodes. In contrast, CD8$^+$ T cells from the spleens of some, but not all cats showed anti-FIV activity. CD8$^+$ T cell-depleted peripheral blood mononuclear cells were cultured and reconstituted with CD8$^+$ T cells on day 12 of culture after confirming FIV replication. The number of FIV proviral DNA copies in the cells did not change, but the amount of FIV p24 antigen production in the culture supernatant and the number of FIV mRNA copies in the cells decreased. These findings suggested that CD8$^+$ T cell anti-FIV activity acts at the level of FIV mRNA synthesis from the FIV proviral DNA, inhibiting FIV replication by a non-cytolytic mechanism.

Introduction

Feline immunodeficiency virus (FIV) was originally isolated in 1986 from a feline leukemia virus (FeLV)-negative cat with chronic opportunistic infections [22]. Based on biological and morphological similarities between FIV and human immunodeficiency virus (HIV), FIV infection of domestic cats is now considered to be an important small-animal model for studying prophylactic and therapeutic

strategies against HIV infections [10, 16, 23, 30]. During the early acute stage of HIV infection, bursts of viral replication and high levels of plasma viremia are observed, then HIV infection enters a long clinically asymptomatic stage [7]. Similar to HIV infection, plasma viremia is detected during the early stage of FIV infection. Thereafter, a long clinically asymptomatic stage continues, during which the number of $CD4^+$ T cells gradually decreases, suggesting the eventual induction of clinical signs of AIDS [6, 17, 33]. The induction of HIV-specific $CD8^+$ cytotoxic T lymphocytes (CTL) is important in maintaining the asymptomatic stage [25]. This CTL is a major-histocompatibility complex (MHC) of a class I molecule-restriction and destroys HIV-infected cells by recognizing the epitopes of Gag, reverse transcriptase (RT), Nef or envelope (ENV) proteins [18]. $CD8^+$ T cells can also control HIV infection without killing the infected cell. This inhibition of HIV replication is noncytotoxic and does not decrease the number of HIV-infected cells [26, 28]. Anti-HIV activity has been demonstrated using a trans-well culture device in which $CD8^+$ T cells were separated from infected $CD4^+$ T cells by a semipermeable membrane, and by exposing infected $CD4^+$ T cells to filtered supernatants from cultured $CD8^+$ T cells [3, 27]. Furthermore, this inhibition can be induced using MHC-mismatched $CD8^+$ T cells [3, 21, 29]. Therefore, the anti-HIV activity of these $CD8^+$ T cells is not due to CTL activity but to soluble factor(s) produced by the $CD8^+$ T cells. Thus, to maintain the asymptomatic stage of HIV infection, the non MHC-restricted noncytotoxic anti-HIV response of $CD8^+$ T cells together with MHC-restricted CTL is important.

As in HIV infection, a viral-specific CTL response was observed following experimental infection with FIV prior to the onset of humoral immunity [1, 24]. In addition, anti-FIV $CD8^+$ T cells with non-CTL characteristics have been observed in FIV-infected cats. We reported that this $CD8^+$ T cell non-cytolytic anti-FIV activity is more effectively induced when $CD8^+$ T cells are in direct contact with the target cells and when $CD8^+$ T cells are induced by autologous $CD8^+$ T cells rather than by allogeneic $CD8^+$ T cells, and that the activity is mediated by soluble factors produced by $CD8^+$ T cells [12]. Jeng et al. [15] detected this $CD8^+$ T cell anti-FIV activity during the asymptomatic stage of FIV infection, but not during the acute stage. However, Bucci et al. [4] and Flynn et al. [8] also detected the activity during the acute stage of FIV infection.

Therefore, to increase understanding of the properties of $CD8^+$ T cell anti-FIV activity, we compared this activity between the period during which it became detectable and during which humoral immunity was detected. We also investigated the presence or absence of this activity in the lymph nodes and spleen. Furthermore, we examined which step(s) of the FIV replication cycle is affected by this antiviral activity by comparing the amount of FIV p24 antigen production, the number of FIV proviral DNA copies and the amount of FIV mRNA produced in the cells.

Materials and methods

Cell culture

Peripheral blood mononuclear cell (PBMC) and CD8$^+$ T cell-depleted PBMC were cultured in RPMI 1640 growth medium supplemented with 10% heat-inactivated fetal calf serum (FCS), antibiotics, 50 μM of 2-mercaptoethanol, 2 μg/ml of polybrene, 100 u/ml of recombinant human IL-2 and 10 μg/ml of concanavalin A (Con A). The medium was replaced with the same medium without Con A every three days.

Experimental animals

Four adult anti-FIV antibody-negative specific pathogen-free cats (Nos. A30, A32, A43, A44) were inoculated with 10^3 TCID$_{50}$ of FIV strain Aomori-II intraperitoneally. Thereafter, PBMC were collected every seven days, and the CD8$^+$ T cell anti-FIV activity was examined. Cats A30 and A44 were killed after 11 weeks, A43 was killed after 13 weeks, and A30 was killed after 22 weeks, and the blood, spleen, and lymph nodes were collected. Single-cell suspensions of the lymph nodes and spleen were prepared by gentle manual homogenization. Mononuclear cells were isolated by centrifugation over lymphoprep (NYCOMED PHAR-MAAS Diagnostics, Norway) and washed three times with Hanks' balanced salt solution (HBSS). Two cats experimentally infected with FIV (KF753 and KF879) were used in the analysis of the mechanism of CD8$^+$ T cell anti-FIV activities. Cats KF753 and KF879 were intraperitoneally injected with 1 ml blood obtained from naturally FIV-infected cat. Both cats were clinically healthy and were in the asymptomatic stage of FIV infection.

Antibodies

CD8$^+$ T cells were depleted and separated using the MAb against human CD8$^+$ T cells, OKT8 (American Culture Collection: ATCC No. CRL8014). This MAb recognizes the α-chain of the human CD8 antigen and crossreacts with feline CD8$^+$ T cells. We also separated CD8$^+$ T cells using magnetic microbeads coated with rat anti-mouse IgG 2a+b (Miltenyi Biotec GmbbH, Germany).

Complement

Blood collected from a rabbit heart was incubated for 1 h at room temperature, then for 1 h at 4 °C. After incubation, the blood was centrifuged at 2,500 rpm at 4 °C for 10 min and complement activity in the rabbit serum was confirmed by hemolytic assays.

Depletion of CD8$^+$ T cells from mononuclear cells

Mononuclear cells from the blood, spleen and lymph nodes (1$\times$10^7 cells) were incubated with 500 μl of purified MAb OKT8 IgG at 4 °C for 30 min, then reacted with eight units of complement in the same volume for 1 h at 37 °C. The cells were then washed three times with HBSS and used as CD8$^+$ T cell-depleted mononuclear cells. The CD8$^+$ T cell-depleted mononuclear cells consistently contained <2% CD8$^+$ T cells.

Separation of CD8$^+$ T cells from PBMC

PBMC (1$\times$10^7 cells) were incubated with 500 μl of purified MAb OKT8 IgG at 4 °C for 30 min. After washing the cells three times with phosphate buffered saline (PBS) containing 2 mM EDTA and 0.5% bovine serum albumin, 40 μl of microbeads coated with rat anti-mouse IgG2a+b (Miltenyi Biotec GmbH, Germany) were added to the cells and the mixture was incubated at 6 °C for 15 min. After washing with PBS containing 2 mM EDTA and 0.5%

BSA, the cells were fractionated into CD8$^+$ T cells and CD8$^+$ T cell-depleted cells using the magnetic system, Mini MACS (Miltenyi Biotec GmbbH, Germany).

p24 FIV antigen assay

Levels of the p24 FIV antigen was measured using a commercial ELISA kit (IDEXX, Portland, ME).

Serological assay

Anti-FIV antibody was measured by an enzyme-linked immunosorbent assay (ELISA) using gradient-purified, disrupted whole FIV antigen. ELISA was performed as described by Hohdatsu et al. [11].

Isolation of DNA preparation from PBMCs

Cellular DNA was extracted from the PBMC pellets using a QIAamp tissue kit (QIAGEN, California, U.S.A.) according to the instructions of the manufacturer.

Quantitative competitive (QC)-PCR primers

A conserved region of the FIV gag gene was amplified by PCR. The Gag QC+ and Gag QC− primers amplified a fragment of either 329 bp (from wild-type FIV) or 277 bp (from the DNA competitor). The primer sequences were as follows: Gag QC+ (nucleotides 1036 to 1055), TATTCAAACAGTAAATGGAG, and Gag QC− (nucleotides 1364 to 1345), CT-GCTTGTTGTTCTTGAGTT.

Competitor synthesis

We synthesized a DNA competitor with a 52-bp internal deletion relative to the wild-type FIV genome was. We designed a sense primer that incorporates the Gag QC+ primer sequence and an additional 12 bp, which targets the region of the FIV genome 52 bp 3′ of the Gag QC+ recognition site, giving a sequence of TATTCAAACAGTAAATGGAGGGCAAGAGAAGG for the primer Gag QC+52. Gag QC− was used as the antisense primer. The template for PCR amplification of the competitor was DNA extracted PBMC infected with the Petaluma strain. The PCR products were cloned in pUC19 plasmid vectors and used as competitive templates in QC-PCR.

QC-PCR

We performed PCR in a total volume of 50 μl. One microliter of sample DNA and 1 μl of a serially diluted competitor DNA template containing from 10^1 to 10^8 copies of the FIV genome were mixed with 5 μl of 10-fold concentrated reaction buffer (Takara, Kyoto, Japan), 5 μl of deoxynucleotide mix (Takara, Kyoto, Japan) containing 2.5 mM of each, 1 μl of 500 mM tetramethylammonium chloride, 2 μl of 20 μM primer mix, 2 μl of Taq polymerase (1 unit/ml; Takara, Kyoto, Japan) and 33 μl of distilled water. The DNA was amplified using a DNA thermal cycler (Atto Co., Japan) after one drop of mineral oil was added to the mixture. Amplification consisted of incubation for 5 min at 94 °C, followed by 30 repeated cycles of denaturation for 1.8 min at 94 °C, primer annealing for 1.8 min at 55 °C, synthesis for 3 min at 72 °C, and a final incubation for 5 min at 72 °C. The PCR products were resolved by electrophoresis on 8% polyacrylamide gels to allow for size separation of the competitor and wild-type products. The gels were incubated with ethidium bromide and bands visualized using a UV transilluminator at 312 nm were photographed. Band intensity was

compared between the competitor and wild-type products. The amount (number of copies) of competitors with which the intensity was equal to that of the wild-type product was regarded as the number of FIV copies in the sample DNA.

Isolation of mRNA preparation from PBMC

Total cellular RNA was extracted from the PBMC pellets using an RNeasy Mini Kit (QIAGEN, California, U.S.A.) according to the instructions of the manufacturer. The RNA preparations were treated for 20 min at 37 °C with 150 U of RNase-free DNase (Takara, Kyoto, Japan) in the presence of 30 U of placental RNase inhibitor (Perkin Elmer, Connecticut, U.S.A.). Cellular mRNA was extracted from total RNA using mRNA kit Oligo [dT]30 (BIO 101 Inc., U.S.A.) according to the instructions of the manufacturer.

Quantification of FIV mRNA by QC-PCR

Using cellular mRNA as a template, cDNA was synthesized using a primer of the FIV gag region (nucleotides 1650 to 1628; TTTTCTTCTAGGGTACTTCTGG). After heating a cellular mRNA solution (7 µl) at 80 °C for 5 min, the solution was immediately transferred into ice. Into this solution, 5 µl of five-fold concentrated first strand buffer (Gibco BRL Life Technologies Inc., Gaithersburg, MD), 0.5 µl of 0.1 M DTT, 10 µl of each 2.5 mM dNTP, 0.5 µl of the primer, 0.5 µl of distilled water, 0.5 µl of RNase inhibitor (10 U; Perkin Elmer, Connecticut, USA), and 0.5 µl of Moloney murine leukemia virus reverse transcriptase (10 U; Gibco BPL Life Technologies Inc., Gaithersburg, MD) were added. The resultant solution was reacted at 37 °C for 3 h, and cDNA was synthesized. Using the synthesized cDNA, the above QC-PCR was performed, and the relative amount of FIV mRNA was determined.

Results

Detection of CD8$^+$ T cell anti-FIV activity induced after FIV infection

The period during which the CD8$^+$ T cell-mediated anti-FIV activity became detectable after experimental FIV infection was investigated. Four SPF cats received an intraperitoneal inoculation of 10^3 TCID$_{50}$ of FIV strain Aomori-II then PBMC were periodically collected. To eliminate CD8$^+$ T cells from the PBMC, the cells were reacted with the MAb, OKT8 and rabbit complement. These CD8$^+$ T cell-depleted PBMC and unseparated PBMC were cultured, and the amounts of FIV p24 antigen produced in the culture fluids were compared to determine the anti-FIV activity of CD8$^+$ T cells. The FIV p24 antigen became detectable in the unseparated PBMC and CD8$^+$ T cell-depleted PBMC at the same level three weeks after FIV inoculation in three cats (No. A32, A43, and A44), and six weeks after inoculation in cat No.A30, showing positivity for FIV isolation. However, a high levels of FIV p24 antigen were produced in the CD8$^+$ T cell-depleted PBMC in cats A32, A43, and A44 at six, seven, and five weeks after inoculation, respectively, indicating that FIV replication is inhibited by CD8$^+$ T cells (Fig. 1). In cat A30, the amounts of FIV p24 antigen slightly differed between the unseparated PBMC and CD8$^+$ T cell-depleted PBMC around 11 weeks after FIV inoculation, but the difference was not significant.

Humoral immune response in cats experimentally infected with FIV

The humoral immune response against the FIV antigens after experimental FIV infection was investigated by ELISA using purified FIV antigens. Figure 2 shows that ELISA values began to increase after five weeks in A32 and A44, after six weeks in A43, and after 11 weeks in A30, confirming that the animals had become anti-FIV antibody-positive.

*Anti-FIV activity of CD8$^+$ T cells from the lymph nodes and spleen*

The anti-FIV activity of CD8$^+$ T cells was examined in mononuclear cells collected from the blood, lymph nodes, and spleen 11 weeks after inoculation in cats A32 and A44, 13 weeks after inoculation in A43, and 22 weeks after inoculation in A30. Figure 3 shows high levels of FIV p24 antigen production in CD8$^+$ T cell-depleted mononuclear cells in the blood, lymph nodes, and spleen from cats A32 and A43, and anti-FIV activity was detected in CD8$^+$ T cells from these animals. High levels of CD8$^+$ T cell anti-FIV activity were detected only in the blood and lymph nodes of cat A44. In A30, the FIV p24 antigen production differed between the unseparated mononuclear cells and CD8$^+$ T cell-depleted mononuclear cells after 15 days of blood culture. After 15 and 18 days of lymph node culture, weak CD8$^+$ T cell anti-FIV activity was detected. However, no anti-FIV activity was detected in the spleen.

Analysis of the CD8$^+$ T cell anti-FIV activity mechanism

We examined which step of the FIV replication cycle is affected by CD8$^+$ T cell antiviral activity. We compared the amount of FIV p24 antigen production, the number of FIV proviral DNA copies and the amount of FIV mRNA in cells from 2 cats.

After experimentally infecting a cat (KF753) with FIV, the PBMC of the cat was cultured by dividing it into two groups (CD8$^+$ T cell-depleted PBMC and unseparated PBMC). Culture supernatant was collected every three days to measure the amount of FIV p24 antigen. Also, cultured cells were collected on days 12 and 15 of the culture to determine the number of FIV proviral DNA copies and the FIV mRNA copies in cells. When the PBMC of the FIV-infected cat was cultured without CD8$^+$ T cells, the amount of FIV p24 antigen in the culture supernatant and the number of proviral DNA copies increased, and the number of FIV mRNA copies in the cells increased at the same time. However, when the unseparated PBMC was cultured, there was no change in the number of proviral

Fig. 1. Detection of CD8$^+$ T cell anti-FIV activity induced after FIV infection. PBMC were divided into CD8$^+$ T cell-depleted PBMC (●) and unseparated PBMC (○), then cultured at 37 °C. Culture supernatants were monitored for FIV p24 antigen at 3-day intervals. Cell cultures were performed in duplicate, and the results were expressed as mean OD values at 650 nm
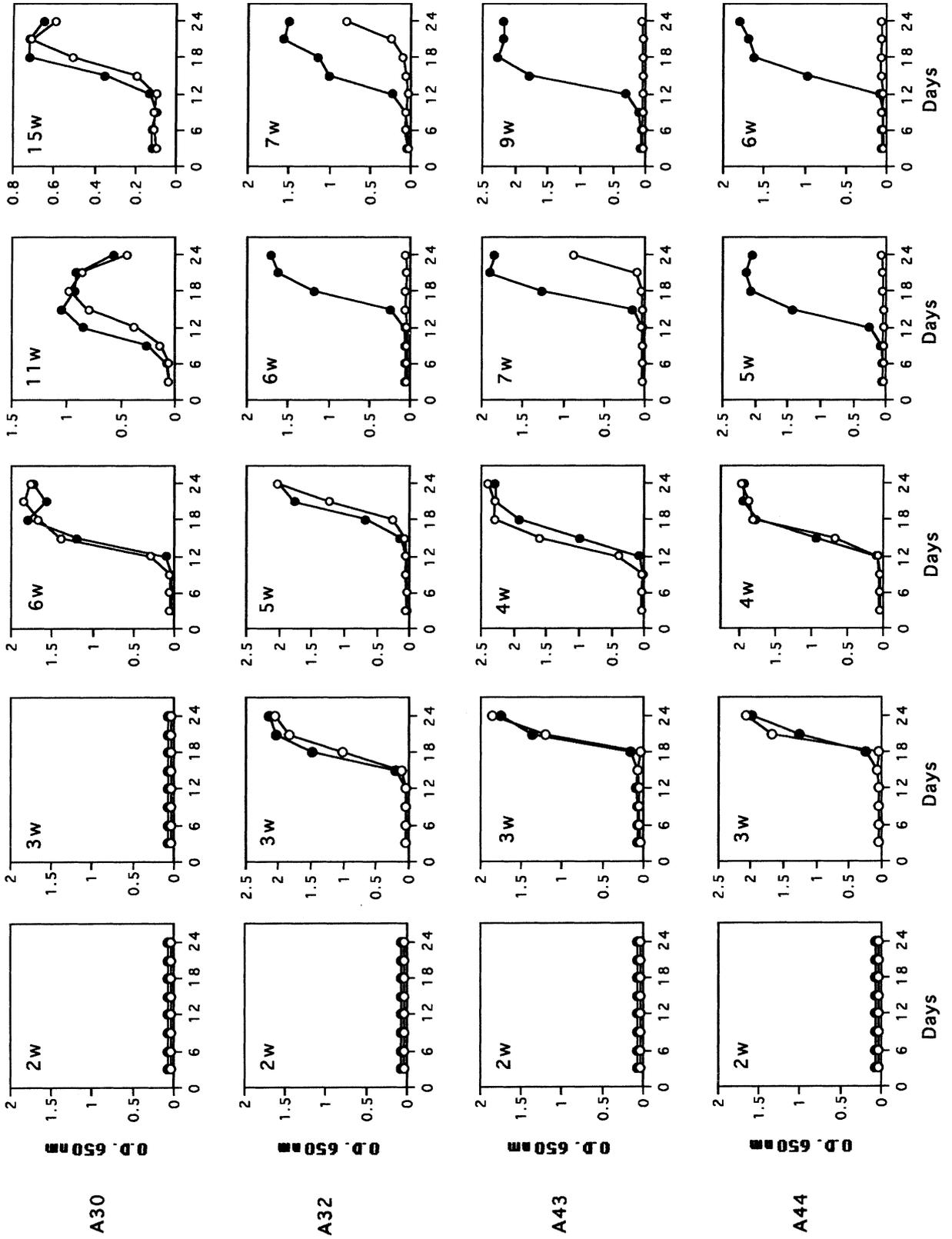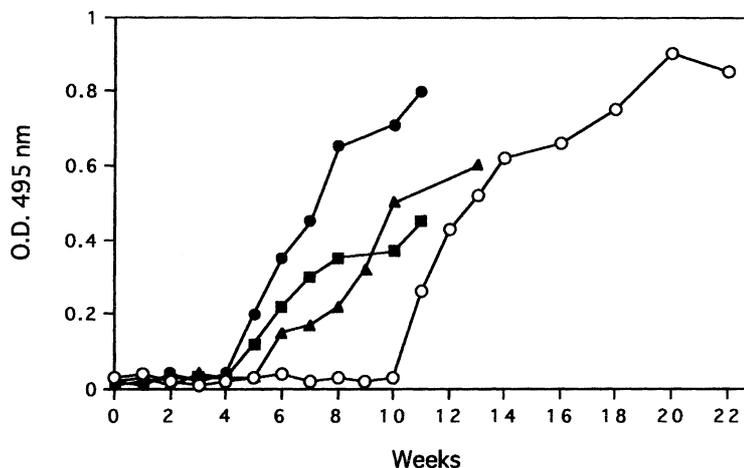
Fig. 2. Humoral immune response of experimentally FIV-infected cats. Anti-FIV antibody was measured by ELISA using gradient-purified, disrupted whole FIV antigen. ○ A30; ■ A32; ▲ A43; ● A44

DNA copies, and the amount of FIV p24 antigen in the culture supernatant and the number of FIV mRNA copies in the cells did not increase (Fig. 4). Therefore, after experimentally infecting a cat (KF879) with FIV, the CD8$^+$ T cell-depleted PBMC of this cat was cultured, and the amount of FIV p24 antigen in the culture supernatant was measured every three days. After 12 days of culture when the FIV p24 antigen began to increase, the cells were divided into two groups. Autologous CD8$^+$ T cells that had been magnetically isolated and activated with mitogen, were added to one of the groups, and the culture was continued. After nine days, the amount of FIV p24 antigen produced in the culture supernatant and the numbers of FIV proviral DNA copies and FIV mRNA copies in the cells were compared between the two groups. The number of FIV proviral DNA copies was not changed regardless of CD8$^+$ T cells, while the amounts of FIV p24 antigen in the culture supernatant and FIV mRNA in the cells were decreased by the CD8$^+$ T cells (Fig. 5).

Discussion

The induction of FIV-specific humoral immune responses such as neutralizing antibody and cell-mediated immune responses such as CD8$^+$ cytotoxic T lymphocyte (CTL) responses is considered to be important for protection against FIV infection [9, 13, 14, 31, 32]. Another cell-mediated immune response (noncytolytic antiviral CD8$^+$ T cells) has recently been implicated in the control of FIV replication during the asymptomatic phase of infection [12, 15]. This anti-FIV activity of CD8$^+$ T cells appears during the acute stage of FIV infection [4, 8]. However, the period during which induction of this activity begins remains unknown. We experimentally infected four SPF cats with FIV, and investigated when the anti-FIV activity of CD8$^+$ T cells appeared. Levels of FIV p24 antigen production were very high in CD8$^+$ T cell-depleted PBMC 5–7 weeks after
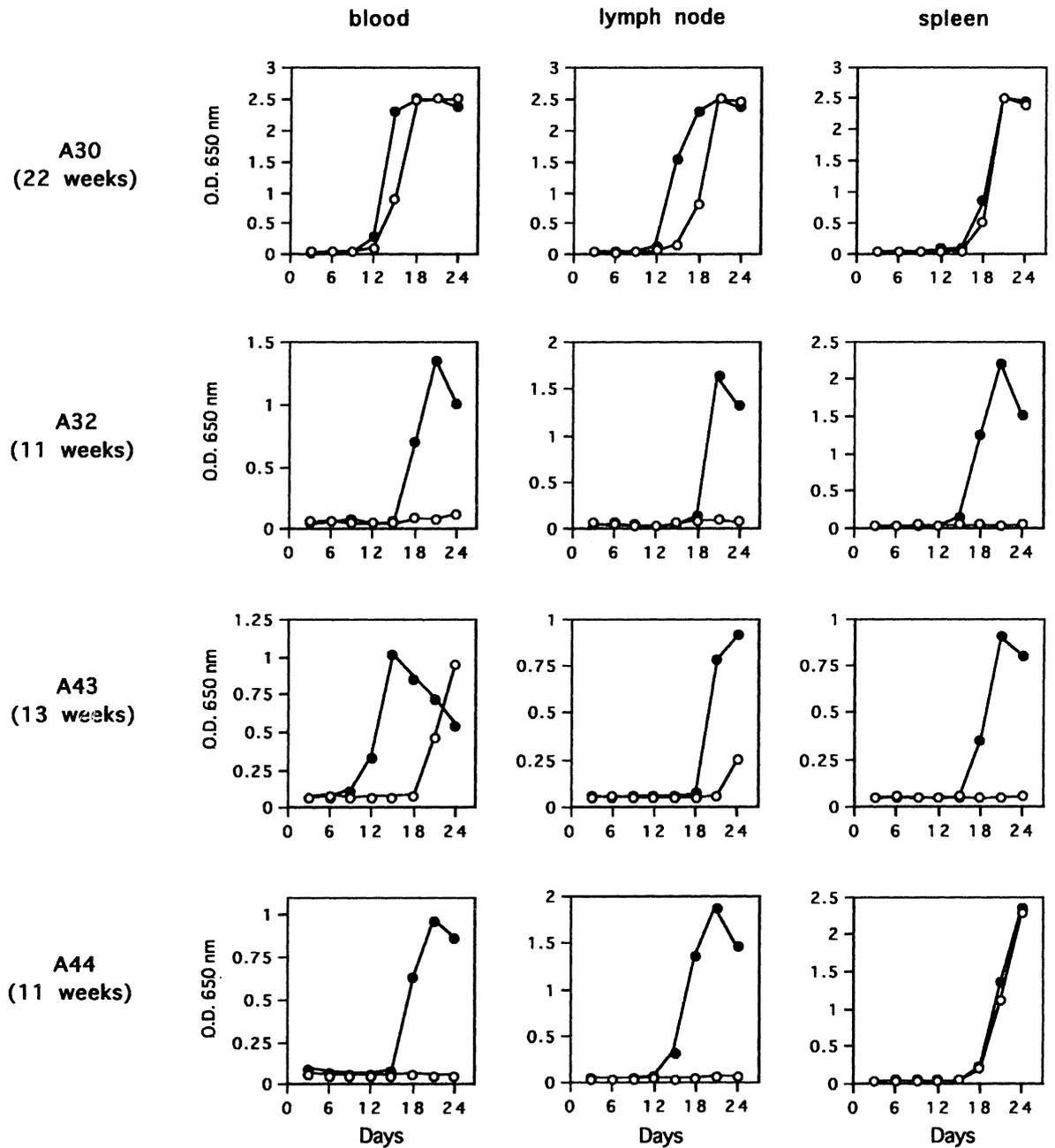
Fig. 3. Anti-FIV activity of CD8$^+$ T cells derived from lymph nodes and spleen cells. After FIV inoculation, mononuclear cells were collected from the blood, lymph nodes, and spleen cells from cats A32 and A44 after 11 weeks, from A43 after 13 weeks, and from A30 after 22 weeks. Anti-FIV activity of CD8$^+$ T cells was examined. Mononuclear cells were divided into CD8$^+$ T cell-depleted (●) and unseparated mononuclear cells (○), then cultured at 37 °C. Culture supernatants were monitored for FIV p24 antigen at 3-day intervals. Cell cultures were performed in duplicate, and the results were expressed as mean OD values at 650 nm
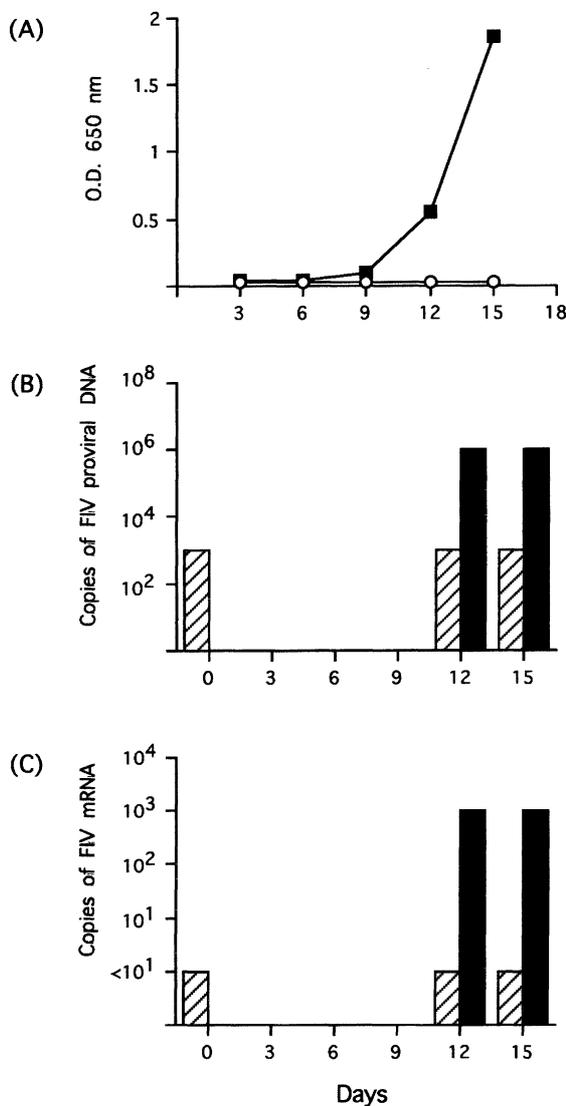
(A)

(B)

(C)

Fig. 4. Changes in the amount of FIV p24 antigen in culture supernatant and numbers of FIV proviral DNA and FIV mRNA copies in cells in unseparated PBMC and $CD8^+$ T cell-depleted PBMC cultures. **A** Unseparated PBMC (1×10^6 cells) and $CD8^+$ T cell-depleted PBMC (1×10^6 cells) were cultured, then FIV p24 antigen levels in culture supernatant were measured every three days. $\bigcirc$ Unseparated PBMC; $\blacksquare$ $CD8^+$ T cell-deplected PBMC. **B** Number of FIV proviral DNA copies in cells. Solid bars, $CD8^+$ T cell-depleted PBMC; Hatched bar, unseparated PBMC. **C** Number of FIV mRNA copies in cells. Solid bars, $CD8^+$ T cell-deplected PBMC; Hatched bars, unseparated PBMC

FIV infection in three of the four infected cats, indicating that FIV replication is inhibited by $CD8^+$ T cells. Humoral immunity became positive 5–6 weeks after inoculation of these cats, which was consistent with the period when anti-FIV activity was induced in $CD8^+$ T cells. In the remaining cat (No. A30), FIV was isolated from PBL six weeks after experimental infection, but humoral immunity was not detected until 11 weeks. Weak $CD8^+$ T cell anti-FIV activity was detected about 11 weeks after inoculation in this cat, but the activity was consistently weak until 22 weeks after inoculation. This phenomenon was considered to be related to a poor immune response including humoral immunity. Anti-HIV activity of $CD8^+$ T cells in HIV uninfected individuals has been reported [20], which may represent crossreactivity with non-HIV antigens. However, the induction of anti-FIV activity of $CD8^+$ T cells was consistent with the induction of
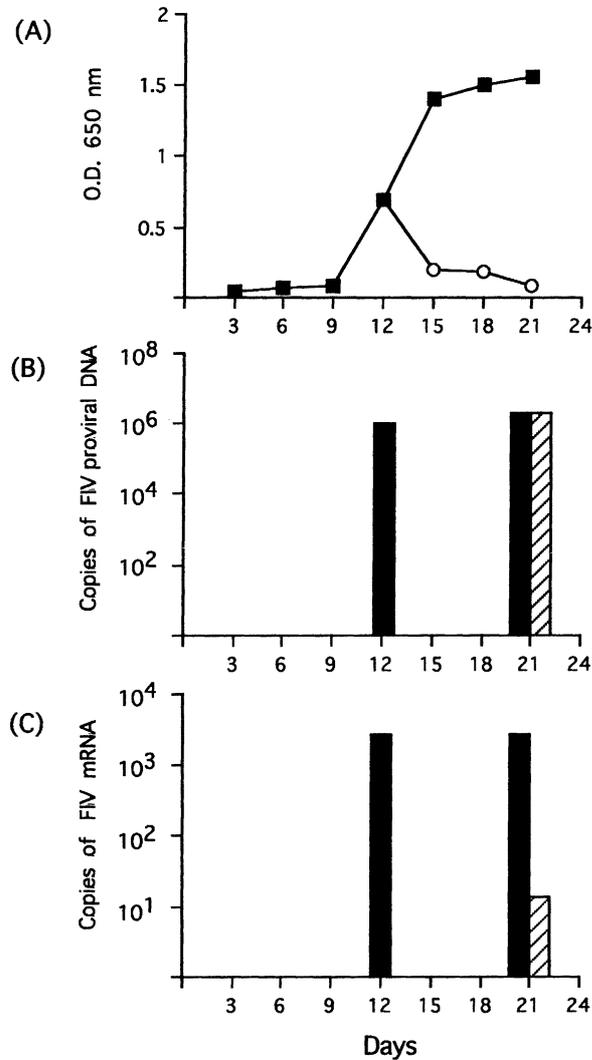
(A)

(B)

(C)

Fig. 5. Changes in the amount of FIV p24 antigen in culture supernatant and numbers of FIV proviral DNA and FIV mRNA copies in cells determined by adding autologous CD8$^+$ T cells to CD8$^+$ T cell-depleted PBMC cultured for 12 days. **A** CD8$^+$ T cell-depleted PBMC (1×10^6 cells) were cultured, then FIV p24 antigen levels in culture supernatants were measured every three days. After 12 days, when FIV p24 antigen levels began to increase, cells were divided into two groups and autologous CD8$^+$ T cells (1×10^6 cells) were added to one of group and cultured. ■ CD8$^+$ T cell-depleted PBMC; ○, reconstituted CD8$^+$ T cell-depleted PBMC with autologous CD8$^+$ T cells. **B** Number of FIV proviral DNA copies in cells. Solid bars, CD8$^+$ T cell-depleted PBMC; Hatched bar, reconstituted CD8$^+$ T cell-depleted PBMC with autologous CD8$^+$ T cells. **C** Number of FIV mRNA copies in the cells. Solid bars: CD8$^+$ T cell-depleted PBMC; Hatched bars: reconstituted CD8$^+$ T cell-depleted PBMC with autologous CD8$^+$ T cells

humoral immunity, suggesting that the activity was induced as an FIV-specific immune response.

CD8$^+$ T cells from the lymphoid tissue of healthy HIV-infected individuals, similar to these cells in the blood, provide substantial control of HIV replication in vitro [2]. The present study, also detected anti-FIV activity in CD8$^+$ T cells in the lymph nodes. Flynn et al. [8] also detected anti-FIV activity in the lymph nodes of FIV-infected cats as well as in the PBMC. They also did not detect this activity in splenic lymphoblasts. They considered that spleen cells in domestic carnivores include high numbers of γ δ T cells, thus explaining the absence of activity in splenic lymphoblasts. However, we detected anti-FIV activity in CD8$^+$ T cells among splenic lymphoblasts in two (A32, A43) of three cats (A32, A43, A44) with high anti-FIV activity in the lymph nodes. Although the ratios of γ δ T cells in spleen cells in these cats were unknown, we confirmed the anti-FIV

activity in CD8$^+$ T cells among spleen cells. Bucci et al. [5] reported that CD8$^+$ T cells with reduced CD8 β chain expression (CD8 α$^+$ β^{low} cell phenotype) have anti-FIV activity. The ratio of CD8 α$^+$ β^{low} cell phenotype in lymph nodes and spleen cells should be investigated.

We examined which step of FIV replication is affected by the anti-FIV activity of CD8$^+$ T cells. The anti-FIV activity of CD8$^+$ T cells was also effective against cells with integrated FIV proviral DNA. Accordingly, this anti-FIV activity affects the step after FIV proviral DNA is integrated into cellular DNA. Therefore, CD8$^+$ T cell-depleted PBL were cultured and CD8$^+$ T cells were added when the level of FIV p24 antigen began to increase in the culture supernatant. We then examined the effects on the amount of FIV p24 antigen produced, the number of FIV proviral DNA copies and the amount of FIV mRNA generated in the cells. To determine the amount of FIV mRNA in the cells, we performed QC-PCR of cDNA synthesized by reverse transcription from mRNA extracted from infected cells and measured the number of copies. Because the efficiency of reverse transcription is not considered in this method, the absolute amount of the original mRNA cannot be estimated, but the relative amount among various samples can be compared. Adding CD8$^+$ T cells to the culture did not change the number of FIV proviral DNA copies, but decreased the FIV p24 antigen level in the culture supernatant and the number of FIV mRNA copies in the cells. These findings suggested that this anti-FIV activity acts at the level of FIV mRNA synthesis from FIV proviral DNA. HIV replication is not inhibited by the CD8$^+$ T cell antiviral activity through blocking viral absorption, but rather through blocking HIV mRNA transcription [19]. Furthermore, the finding that the number of FIV proviral DNA copies was not decreased despite decreases in the amount of FIV p24 antigen and the number of FIV mRNA copies supported the notion that the anti-FIV activity of CD8$^+$ T cells is induced by a non-cytolytic mechanism.

CTL typically recognize viral antigens expressed on the surface of infected cells in the context of an appropriate MHC molecule and kill these cells by lysis or apoptosis, and this response plays a central role in eliminating virus-infected cells from the body. The non-cytolytic anti-FIV activity of CD8$^+$ T cells decreased the expression of viral antigens, which interfered with the beneficial CTL activity. Considering the effect of this non-cytolytic response on CTL activity, this anti-FIV activity of CD8$^+$ T cells may confuse its role in eliminating the virus from the body in FIV-infected cats. However, HIV-specific CTL are largely involved in a decrease in CD4$^+$ T cells, and the harmfulness of CTL has been noted in the pathogenesis of AIDS [34]. To prevent the onset of AIDS, a dynamic balance between the virus and target cells must be maintained. In other words, HIV/FIV replication should be maintained at low levels. Vigorous viral replication induces vigorous viral mutation. By suppressing viral replication in the cell, CD8$^+$ T cells would prevent mutations that could lead to highly cytopathic strains, that may play a role in FIV pathogenesis.

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