The acute stage of feline immunodeficiency virus (FIV) infection is characterized by a CD8$^+$ anti-FIV response that parallels the appearance of a CD8$^+$ subpopulation with reduced expression of the $\beta$ chain (CD8$\alpha^+$ $\beta^+$). The relationship between the CD8$\alpha^+$ $\beta^+$ phenotype and CD8$^+$ anti-FIV activity was examined. Flow cytometric analysis of peripheral blood mononuclear cells with anti-CD8$\beta$ chain monoclonal antibody 117 revealed that the CD8$\alpha^+$ $\beta^+$ phenotype expanded throughout the asymptomatic infection, constituting 80%–90% of the CD8$\beta^+$ cells in long-term–infected cats. Purified CD8$\alpha^+$ $\beta^+$ and CD8$\alpha^+$ $\beta^+$ subpopulations were analyzed for anti-FIV activity in an acute infection assay. Anti-FIV activity resided principally in the CD8$\alpha^+$ $\beta^+$ population and was demonstrated in acute FIV infections, as well as in long-term asymptomatic infections. These data suggest that a unique CD8$\alpha^+$ $\beta^+$ anti-FIV phenotype arises early in infection and may play a major role in eliminating virus and maintaining the asymptomatic infection.

Feline immunodeficiency virus (FIV), similar to human immunodeficiency virus (HIV), induces a CD8$^+$ T cell lymphocytosis during the acute-stage infection that persists throughout the asymptomatic stage of disease [1–3]. In both infections, the CD8$^+$ lymphocytosis correlates with the emergence of a population of CD8$^+$ cells with potent antiviral activity. The CD8$^+$ cells capable of limiting viremia have been classified by the means by which they mediate antiviral activity. In addition to classic major histocompatibility complex (MHC)–restricted cytotoxic lymphocytes (CTL) [4], a population of effector cells that suppress viral replication through non–MHC-restricted, noncytotoxic mechanisms has been described for HIV [4–7] and FIV [8, 9].

The importance of CD8$^+$ cell–mediated immunity in controlling viral replication is underscored by the observations that a decrease in acute-stage viremia correlates with a CD8$^+$ anti-HIV response [10, 11]. In addition, many long-term–nonprogressor HIV-infected patients maintain elevated levels of CD8$^+$ cells, while decreasing numbers of CD8$^+$ cells correlate with increased viremia and disease progression [12, 13]. Additionally, several groups have reported a correlation between a strong CD8$^+$ anti-HIV response and the apparent clearance of HIV from the circulation, in the absence of an antibody response [14, 15]. We have similarly demonstrated cases of sero-negative FIV-exposed cats in which virus was cleared from the circulation in the face of a strong acute-stage CD8$^+$ anti-FIV response [9]. In contrast, FIV replication was poorly controlled in cats displaying weak or no CD8$^+$ antiviral activity [9]. These observations offer a compelling argument for the importance of a CD8$^+$ cell–mediated immune response in the control, and perhaps elimination, of virus following primary HIV and FIV infections.

Studies designed to define the surface phenotype of these effector cells suggest that surface markers on CD8$^+$ antiviral cells may provide differential prognostic indicators of disease progression. Landay et al. [13] demonstrated that HLA-DR$^+$ and CD38$^+$ expression on CD8$^+$ lymphocytes correlates with high antiviral activity. However, it was also reported that the CD8$^+$ CD38$^+$ phenotype was associated with high viremia and provirus burden and disease progression [2, 16]. In contrast, long-term–nonprogressor patients possessed high numbers of HLA-DR$^+$ CD38$^-$ CD8$^+$ cells [17]. Interestingly, the HLA-DR$^+$ CD38$^+$ CD8$^+$ phenotype that predominates in long-term–nonprogressor patients is noncytotoxic, raising the possibility that these cells reduce viremia by a suppressive mechanism. A subset of CD8$^+$ cells expressing the CD28 molecule has been reported to be primarily responsible for suppression of HIV replication in CD4$^+$ cells [18]. Moreover, Barker et al. [18] demonstrated that CD28 costimulation with alloglycocy- anin (APC)-conjugated monoclonal antibody (MAb) B7 increased CD8$^+$ cell–mediated suppression of HIV, suggesting that intracellular signals induced by B7-CD28 engagement play a role in HIV suppression.

Similar surface phenotypic analysis has not been done with CD8$^+$ cytotoxic or virus-suppressive cells in FIV-infected cats. Although Lehmann et al. [19] and Willett et al. [20] reported...
an early expansion of a CD8+ subpopulation with reduced expression of the CD8 molecule in FIV-infected cats, neither group attributed any function to these CD8+ cells. The two anti-CD8 MAbs, FT-2 [19] and VPG-9 [20], used to demonstrate down-regulation of cell surface CD8 were subsequently shown to be specific for the CD8 α chain and the CD8 α/β complex, respectively [21], suggesting that FIV causes a selective decrease in surface expression of the CD8 β chain. Shimojima et al. [22] confirmed the presence of CD8α+/β− cells in FIV-infected cats and also described the expansion of a CD8α+/β− subpopulation in long-term–infected cats.

As we previously reported that CD8+ cell antiviral activity was expressed during the acute-stage infection and was sustained through the asymptomatic infection [8, 9], we explored the possibility that CD8+ anti-FIV activity could be mediated by the phenotype with reduced surface expression of the CD8 β chain (CD8α+/β−).

Materials and Methods

Animals and infection. For acute-stage FIV infection studies, specific pathogen–free cats ~6 months of age were obtained from Liberty Laboratories (Liberty Corners, NJ). One group of 4 cats was infected intravenously (iv) with 6.0 × 10^6 TCID50 of the NCSU; FIV isolate as described previously [23]. A second group of 3 cats was infected by intravaginal administration of bronchoalveolar lavage (BAL) cells infected with FIV NCSU1. BAL cells consisting predominantly of alveolar macrophages (80%) were collected from normal cats by previously described methods [24], washed three times in PBS, and placed in Dulbecco’s MEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. After 24 h, cells were washed, incubated in fresh medium with lipopolysaccharide (5 μg/mL), and infected with FIV NCSU1 at an MOI of 0.4 TCID50. Twenty-four hours after infection of BAL cells, cells were sedated with ketamine (20 mg/kg), and 5.0 × 10^6 infected BAL cells were deposited in the anterior vagina in a volume of 100 μL of medium. Inoculum discharge from the vulva was not observed. As a precaution, wide plastic Elizabethan collars were placed on the cats for 72 h to prevent the cats from grooming the perineal area.

For chronic FIV infection studies, 3 specific pathogen–free cats (Liberty Laboratories) infected with FIV from 6 months to 7 years before this study were used. These long-term FIV-infected cats have been described previously [1] and were seropositive for FIV antibodies and positive by polymerase chain reaction for FIV provirus but clinically asymptomatic at the time of this study. Control cats for the chronic FIV infection studies ranged from 1 to 3 years of age.

Flow cytometry. Lymphocyte subset analysis was done on peripheral blood collected by jugular venipuncture into citrated tubes. Two-color flow cytometric analysis as described by Davidson et al. [23] was used to determine the presence of CD4+, CD8+, and B cell populations. CD8+ cells were monitored for the development of distinct subpopulations on the basis of fluorescence intensity of staining by antibodies specific for the CD8 α or β chain. Classification of these CD8+ subpopulations was done with MAB 117 (anti-CD8 β chain) and MAB 357 (anti-CD8 α chain) developed in our laboratory [25], as well as MAB FT-2 (anti-CD8 β chain) [26] and MAB VPG-9 [20] (anti–CD8 α/β complex) developed in other laboratories.

For analysis of CD8 α chain and CD8 β chain expression on CD8+ cells, antibody blocking studies were done. CD8+ cells were first incubated with saturating levels of unlabeled MAB and then counterstained with fluorochrome-labeled MAB of known CD8 α and β chain specificity. The MABs used in these studies were MAB 117 conjugated to APC; MAB 357 conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE), FT-2 conjugated to FITC, and VPG-9 (unlabeled). In addition, two-color flow cytometry colocalization studies with anti–CD8 α chain and anti–CD8 β chain MAB labeled with different fluorochromes were done. Data were acquired on a FACSCalibur or FACSscan (Becton Dickinson, San Jose, CA). Samples stained with APC were acquired on the FACSCalibur, using a helium-neon laser as the second excitation source. For all samples, data from at least 15,000 cells were acquired and stored list-mode fashion for subsequent analysis. For samples in which the absolute CD8+ cell number was low, data from at least 30,000 cells were collected to provide a minimum of 4000 CD8+ cells for subsequent analysis. Gated data were then generated for fluorescent analysis of lymphocytes as defined by forward and side scatter.

Assessment of CD8+ cell inhibition of FIV replication in autologous peripheral blood mononuclear cells (PBMC) by CD8+ cell depletion assay. Anti-FIV activity mediated by CD8+ cells was monitored by a standard CD8+ cell depletion assay using anti-CD8 MAB–coated magnetic beads as described [9]. For CD8+ cell depletion, 3 × 10^6 Percoll-separated PBMC were incubated with magnetic beads precoated with goat anti-mouse IgG (M450; Dynal, Lake Success, NY) and treated with anti-CD8 MAB 357 at a bead-to-cell ratio of 3:1 as described [9]. The cell-bead mixture was incubated with agitation at 4°C for 1 h and magnetically sorted to obtain CD8+ cell–depleted PBMC. The CD8+ cell–depleted PBMC were analyzed by flow cytometry for distribution of CD4+, CD8+, and B cell phenotypes by two-color flow cytometry analysis [25]. This separation technique routinely yields ≥98% CD8+ cell depletion. Unfractionated PBMC (1 × 10^5) or CD8+ cell–depleted PBMC adjusted to contain the same number of non-CD8+ cells as in the unfractionated PBMC were cultured in the presence of feline CD4+ lymphocytes (FCD4E) (1 × 10^5/well) in the presence 100 U/mL recombinant human interleukin-2 (Biological Resource Branch, NIH, Bethesda, MD). Cell culture supernatants were assayed after 10 days for FIV p26 by ELISA (Idexx, Portland, ME). Ten days in culture was chosen, as Bucci et al. [9] previously reported this to be the time of peak p26 production in FIV-infected FCD4E cells cultured alone.

Assessment of FIV inhibition by CD8α+β+ and CD8α+β+ subpopulation. To assess the relative antiviral activity of CD8+ cell subpopulations, a modified version of an acute infection assay routinely used for HIV infection was used [13, 27]. FCD4E cells (5 × 10^5) were infected with FIV NCSU1, at an MOI of 0.1 for 18 h. This dose of virus yields a productive infection of FCD4E cells with syncytium formation and high levels of p26 by 10 days after infection. After 18 h, the FIV-infected target cells were washed twice with RPMI 1640 and cocultured in triplicate with FACSter Plus (Becton Dickinson)–sorted CD8α+β+ or CD8α+β+ cells at CD8+ to-FCD4E cell ratios ranging from 1.0 to 0.1. As a positive control, infected FCD4E cells were cultured in the absence...
of effector CD8+ cells. CD8+ cells from normal cats served as an additional control. After 10 days of culture, cell supernatants were assayed for the presence of FIV p26 by ELISA (IDEXX). Inhibition of FIV production was expressed as a reduction of p26 optical density in the presence of CD8+ cells.

Statistical analysis. Data were analyzed by a paired t test to determine differences between means. All data are presented as mean ± SD.

Results

**MAb 117 binds to the β chain of the CD8 molecule.** The CD8 molecule is a heterodimeric protein composed of an α and β chain. Shimojima et al. [22] recently reported that expression of the CD8 β chain but not the CD8 α chain was lost or markedly reduced on a subpopulation(s) of CD8+ cells in FIV-infected cats. We have previously observed that anti-CD8 MAb 357 frequently detected greater numbers of CD8+ cells in FIV-infected cats than did anti-CD8 MAb 117. As Shimojima et al. [22] demonstrated that MAb 357 reacted specifically with the cloned CD8 α chain, we speculated that MAb 117 may be specific for the CD8 β chain. To address this question, colocalization and antibody-blocking studies were done using MAbs with known specificities for α or β chains [22] or the α/β complex [22]. Cell cytometric analysis revealed that pretreatment of cells with saturating concentrations of unlabeled MAb 117 completely blocked the binding of FITC-labeled CD8 β chain-specific MAb FT-2 (figure 1A), whereas pretreatment with unlabeled MAb 357 (anti–CD8 α chain) had no effect on the binding of FITC-labeled FT-2 (figure 1B). Also, pretreatment of cells with unlabeled MAb VPG-9 (anti-CD8 α/β complex) had no effect on binding of APC-labeled MAb 117 (figure 1C). We also observed that pretreatment with MAb 117 does not affect binding of FITC-labeled MAb 357 and VPG-9 (data not shown). Two-color cell cytometric analysis with MAb 117 (APC) and 357 (FITC), or FT-2 (FITC) and 357 (PE), showed that MAb 117 and FT-2 bind to the same population of CD8+ cells (figure 1D, E). Thus, we conclude that MAb 117 binds specifically to the CD8 β chain.

**Decreased expression of CD8 β chain in FIV-infected cats.** Several laboratories [19, 20, 22] have described a subpopulation of CD8+ cells with decreased surface expression of the CD8 β chain in FIV-infected cats. To determine if the CD8α+βlo phenotype was also associated with the FIV NCSU isolate, CD8 density on PBMC from normal and asymptomatic FIV NCSU–infected cats was evaluated by flow cytometry using anti–CD8 β chain MAb 117. The FIV-positive cats were infected iv with FIV NCSU; 6 months to 7 years before this study and were clinically asymptomatic, although seropositive for FIV antibodies at the time of this study. Control cats ranged in age from 1 to 3 years. The histograms in figure 2 demonstrate typical patterns of CD8 β chain expression on PBMC from normal and FIV-infected cats. In the case of normal cats, MAb 117 binding to CD8+ cells yields a single sharp peak of relatively high fluorescence intensity (figure 1A). In contrast, CD8 β chain expression on CD8+ cells from FIV-infected cats was bimodal (figure 2B, C), suggestive of two distinct subpopulations of CD8+ cells. The FIV-associated CD8α+βlo phenotype was indicated by a leftward shift in fluorescence intensity of a major subpopulation of CD8+ cells. The CD8α+βlo subpopulation represented >80%–90% of the total MAb 117–positive CD8+ cell population in the cat infected for >7 years (figure 2C).

Using the distinct bimodal distribution of MAb 117 epitope on CD8+ cells, we analyzed a large number of normal and FIV-infected cats for distribution of CD8α+βhi and CD8α+βlo subsets. Cell cytometric analysis of a group of normal and FIV-infected cats revealed that normal cats maintain an arithmetic mean population of CD8α+βlo cells of ~24% of total CD8+ cells (figure 3). In contrast to the case in control cats, ~50% of CD8+ cells from cats with acute-stage FIV infection (<6 months after infection) were of the CD8α+βlo phenotype and ~15% of CD8+ cells were CD8α+βhi in infected cats for 6–18 months. In those cats with long-term asymptomatic FIV infections (>7 years), the MAb 117–positive cells were >80% CD8α+βlo phenotype (figure 3). Thus, there appears to be a progressive expansion of the CD8α+βlo subpopulation throughout the asymptomatic stage of FIV infection that persists in the long-term–infected cats. Analysis of CD8 α chain expression by use of anti-CD8 α chain–specific MAb 357 did not show a similar reduction in expression of the CD8 α chain (data not shown), as was also reported by Shimojima et al. [22].

**Temporal development of the CD8α+βlo phenotype during acute-stage FIV infection.** To determine the temporal response of the CD8+ phenotype in the early acute stage of FIV infection, CD8α+βlo cells were evaluated by cell cytometric analysis following iv FIV infection. Expansion of the CD8α+βlo phenotype was evident as early as 2–3 weeks after FIV infection (figure 4). Only 1 cat was seropositive at 3 weeks after infection, whereas all 4 cats were antibody-positive at 4 weeks after infection by ELISA. Thus the CD8α+βlo phenotype emerges in some cats before seroconversion and increases in number during the acute-stage infection.

**Anti-FIV activity is mediated by the CD8α+βlo phenotype in acute-stage FIV-infected cats.** Three of the iv-infected cats (RJ2, QZ4, and RN7) shown in figure 4 and 3 cats (YP2, KT1, and KJ6) challenged by intravaginal inoculation of FIV-infected BAL cells 6 weeks previously were analyzed for anti-FIV activity by the CD8+ cell depletion assay [9]. The fourth cat in the iv infection group was lost because of a sampling error. Figure 5 shows that 5 of 6 cats had CD8+ anti-FIV activity as determined by the PBMC assay, as indicated by increased FIV p26 production in PBMC depleted of CD8+ cells. The percentage of CD8α+βlo cells in these 6 cats ranged from 48% to 72% and there was no strict correlation between the percentage of CD8α+βlo phenotype and the antiviral activity. Although the number of cats is limited, these data suggest that the route of FIV infection does not appear to influence the development of CD8+ cell–mediated anti-FIV activity.
Figure 1. Monoclonal antibody (MAb) 117 binds epitope on feline CD8 $\beta$ chain. Whole blood lysates were prepared for cell cytometric analysis and reacted with MAb of known specificities for feline CD8 $\alpha$ chain (357), $\beta$ chain (FT-2), or $\alpha/\beta$ complex (VPG-9) in antibody blocking studies. Shown are flow cytometric histograms of lymphocytes reacted with (A) fluorescein isothiocyanate (FITC)-labeled FT-2 alone (stippled line) or unlabeled MAb 117 followed by FT-2–FITC (solid line); (B) FT-2–FITC alone (stippled line) or unlabeled MAb 357 followed by FT-2–FITC (solid line); (C) allophycocyanin (APC)-labeled MAb 117 alone (stippled line) or unlabeled VPG-9 followed by 117–APC (solid line). Also shown are bivariate displays of 2-color analysis of lymphocytes reacted with (D) 357-FITC and 117-APC or with (E) phycoerythrin (PE)-labeled 357 and FT-2–FITC.

When the MAb 117 staining on the PBMC from cats described in figure 3 was analyzed as mean index of fluorescence, we found that the CD8$\alpha^+\beta^-$ subpopulation was 3.6 times brighter than the CD8$\alpha^-\beta^+$ subpopulation. This allowed us to purify these two subpopulations by FACStar Plus sorting on the basis of their respective mean index of fluorescence using FITC-conjugated MAb 117. The resulting population was >98% pure for either CD8$\alpha^+\beta^-$ or CD8$\alpha^-\beta^+$. To determine if the anti-FIV CD8$^+$ cell activity was mediated by the CD8$\alpha^-\beta^+$ cells, flow-sorted CD8$\alpha^-\beta^+$ and CD8$\alpha^+\beta^-$ subpopulations were tested for antiviral activity against FIV-infected FCD4E cells in the acute infection assay described above. As shown in figure 6A, CD8$\alpha^-\beta^+$ cells from 4 of the 6 cats had strong antiviral activity against the heterologous FIV-infected FCD4E targets. These 4 cats were also positive in the CD8$^+$ cell depletion assay (figure 5). CD8$\alpha^+\beta^-$ cells from FIV-positive cats and total CD8$^+$ cells from control cats showed no evidence of significant antiviral activity in the heterologous FCD4E cell culture system (figure 6A).

Cats RJ2 and YP2 did not fit the expected pattern of CD8$\alpha^-\beta^+$ virus suppression. Cat RJ2 has very low levels of virus production in both assays, suggesting low PBMC virus burden. It is possible that CD8$\alpha^+\beta^-$ cells reduced virus burden before the assay 6 weeks after infection and were no longer activated when assayed at that time point. The fact that CD8$\alpha^-\beta^+$ cells from cat YP2 demonstrated stronger anti-FIV activity in the CD8$^+$ cell depletion assay (figure 5) than in the in vitro FCD4E infection assay (figure 6A) may be due to
discrepancies. ral cells were effectively controlling FIV replication in vivo indicated by the observation that the anti-FIV response mediated by the CD8

*Discussion*

Lehman et al. [19] and Willett et al. [20] reported an early and sustained expansion in the blood of FIV-infected cats of a subset of CD8 T cells with reduced surface expression of the CD8 molecule. Shimojima et al. [22] subsequently demonstrated that this phenotype reflected a selective reduction in surface expression of the CD8 beta chain (CD8αβlo). In this study, we confirmed these observations and further demonstrate that the CD8αβlo subset of T cells may be the dominant phenotype responsible for the previously described CD8+ cell--mediated immunity to FIV that develops during the acute stage of infection [8, 9].

Antibody-blocking and colocalization studies with MAb of known CD8 alpha and beta chain specificities demonstrated that MAb 117, developed in our laboratory, bound specifically to the CD8 beta chain. Using MAb 117 and MAb 357 (anti--alpha chain), we were able to demonstrate that the CD8 beta chain but not the CD8 alpha chain was markedly reduced on the surface of CD8+ cells from the blood of FIV-infected cats. Cell cytometric analysis with MAb 117 demonstrated that the CD8αβlo phenotype increases in some cats before seroconversion, increases in number during acute infection, and becomes the predominant CD8+ cell population in asymptomatic, long-term--infected cats. In cats infected for ≥7 years, the CD8αβlo subset constituted 80%–90% of the total CD8β+ cell population in the peripheral blood. Willett et al. [20] also reported a rapid expan-
from circulation by 2 months after infection. This is consistent with the time frame of CTL responses described for numerous viral infections [29]. In these cases, the decline in circulating CD8^+ effector cells presumably correlates with elimination of the infectious agent.

If the CD8^a/b^lo phenotype in FIV-infected cats is an effector T cell, its sustained presence over a period of years is an unusual homeostatic condition. It is possible that the persistent activation of the CD8^a/b^lo phenotype is the result of chronic exposure of CD8^+ cells to FIV antigens in lymph nodes or other tissue. Although virus expression is low in peripheral blood and other tissues during the asymptomatic stage of infection, infectious virus and viral antigens can be detected in some asymptomatic infected cats. There are no data to indicate whether low levels of virus during the asymptomatic stage of FIV infection are due to rapid virus turnover, as has been suggested for HIV infection [30, 31], or due to low levels of virus production by infected cells. However, chronic viremia does not appear to be the explanation for sustained expression of the CD8^a/b^lo phenotype, as Willett et al. [20] demonstrated that cats in the viremic stage of feline leukemia virus infection failed to develop increased numbers of CD8^a/b^lo cells. This is consistent with our observation that the CD8^a/b^lo phenotype appears to be FIV infection–specific, as viremic feline leukemia virus–infected cats, as well as clinical cases presented at

Figure 3. Expression of CD8^a/b^lo phenotype throughout course of experimental FIV infection. Peripheral blood mononuclear cells from cats infected with FIV for different durations were analyzed by cell cytometry for high or low expression of CD8^beta chain with fluo-

erescein isothiocyanate–conjugated monoclonal antibody (MAb) 117. Duration of FIV infection is depicted on x axis (seronegative, n = 19 cats; 1–6 months after infection [PI], n = 20 cats; 6–18 months PI, n = 20 cats; >60 months PI, n = 8 cats), and y axis represents % of CD8^a/b^lo cells in total CD8^+ population recognized by MAb 117. Box plot consists of 5 data points with top, bottom, and line running through center of box corresponding to 75th, 25th, and 50th percentiles, respectively; bars represent 10th and 90th percentiles. "X" indicates arithmetic mean for number of cats for each group.

Figure 4. Kinetics of development of CD8^a/b^lo phenotype during acute stage of FIV infection. Cats were infected intravenously with FIV NCSU1 and bled at weekly intervals (x axis) for analysis of CD8^beta chain expression by flow cytometry with monoclonal antibody 117. 2 uninfected cats served as controls. y axis represents % of CD8^a/b^lo cells present.
the North Carolina State University Veterinary Medicine Clinic with a variety of infectious diseases, did not have numbers of CD8α−βlo cells beyond that of control cats (data not shown). While CD8− anti-HIV cells with increased expression of the MHC class II molecules and other activation markers have been reported in HIV-infected patients [17, 18], to the best of our knowledge CD8α−βlo cells have not been reported in these patients. As pointed out by Shimojima et al. [22], CD8α+ cells may have been overlooked in HIV-positive patients, as most phenotypic analysis of human CD8+ cells uses MAb OKT8, which detects the CD8α chain. We and others [22] have demonstrated that the CD8α chain is not significantly reduced on CD8− cells in FIV-infected cats.

While we do not yet understand the significance of CD8β chain loss, it could have a profound effect on antigen-specific CTL activity, as the CD8 molecule functions as a necessary coreceptor for CTL recognition of MHC class I–associated antigen on antigen-presenting cells as well as on virus-infected target cells. In this regard, Baume et al. [32] described CD8α−β cytotoxic cells in humans that were not MHC-restricted. In addition, Moebius et al. [33] demonstrated that anti-CD8 antibodies blocked cytotoxicity of CD8α−β CTL but not that of CD8α+ cells, suggesting the latter did not utilize CD8 in target cell binding and were not MHC-restricted. Shimojima et al. [22] described a subpopulation of CD8α+ cells in long-term–infected cats; however, no function was attributed to them. We have also observed a major population of CD8α+ cells in cats with long-term FIV infections (data not shown). Whether the CD8α−β phenotype represents a progressive loss of the CD8β chain from the CD8α+ βlo phenotype or a distinct phenotype is not known.

Despite the marked reduction in the CD8β chain on the CD8α−βlo cells in FIV-infected cats, they possess potent antiviral activity against FIV–infected CD4+ cells. We reported previously that FIV infection of cats results in a strong CD8+ cell response that inhibits FIV replication in autologous in vivo–infected PBMC [8, 9]. CD8+ anti-FIV activity was detected as early as 6 weeks after infection and correlated with decreased plasma- and PBMC-associated viremia [9]. In this study, we demonstrated that at least a proportion of this anti-FIV activity is mediated by the CD8α−βlo phenotype that also emerges during the acute-stage infection. We used the marked difference in mean index of fluorescence of FITC-labeled MAb 117 for CD8α+ cells and CD8α+ cells to obtain highly enriched populations of the two phenotypes and showed that the CD8α+ βlo subset strongly suppressed FIV replication in acutely infected heterologous FCD4E (CD4+) cells.

Because of the long-term (10-day) assay required for antiviral effects of CD8α−β cells, these experiments do not distinguish intrinsic anti-FIV activity from effector function that could have been induced during the effector-target coculture. Additionally, antiviral effects mediated by CD8α−βlo cells may be regulated by cytokine or chemokine production or other factors that were not investigated in this study. Certainly these immunologic molecules may effect the activation state of the CD8α−βlo cells as well as infection of target cells with FIV. While we did not observe a similar anti-FIV response by total CD8+ cells from uninfected control cats, it will be important to examine purified CD8α−βlo cells from control cats in this assay.

Similar to our studies with FIV, CD8+ cells from HIV-infected patients have the ability to suppress HIV replication in vivo–infected CD4+ cells [34] and in vitro–infected heterologous or allogeneic [35] CD4+ cells. In the case of HIV, this antiviral activity against HIV-infected allogeneic cells is noncytotoxic, is not restricted by MHC, and does not require
Figure 6. Anti-FIV activity of CD8α⁺βlo and CD8α⁺βhi cells in feline CD4⁺ lymphocyte (FCD4E) acute FIV infection assay. A, peripheral blood mononuclear cells were purified on basis of relative mean index of fluorescence by flow cytometry into CD8α⁺βhi and CD8α⁺βlo subpopulations (≥98% purity) with monoclonal antibody 117. Subpopulations were cocultured with acutely infected FCD4E cells (effector-to-target ratio, 0.5) for 10 days. As control, infected FCD4E cells were cultured in absence of CD8⁺ effector cells or with total CD8⁺ cells from uninfected cats (GX4 and DL6). Culture supernatants were assayed for FIV p26 production after 10 days of coculture by ELISA. Bars represent SD of mean of triplicate samples. * CD8α⁺βlo and CD8α⁺βhi cocultures are significantly different (P < .005) by paired t test. B, Purified CD8α⁺βlo and CD8α⁺βhi subpopulations from single representative cat (RN7) were incubated at different effector-to-FCD4E cell ratios. Data demonstrate dose-dependency of antiviral response mediated by CD8⁺βlo cells at effector-to-FCD4E target cell ratios ranging from 1.0 to 0.1. * CD8α⁺βlo cocultures and FCD4E control are significantly different (P ≤ .005).

Contact between target and effector cells [4]. In the absence of knowledge on the polymorphism of feline class I MHC, we cannot draw any conclusions as to whether the CD8α⁺βlo effector activity against FIV-infected PBMC or FCD4E (CD4⁺) cells is mediated by an MHC-restricted cytotoxic mechanism or a non-MHC-restricted virus-suppressive mechanism. Jeng [36] presented preliminary evidence that supernatants from concanavalin A–stimulated CD8⁺ cells with anti-FIV activity suppressed FIV replication in acutely infected FCD4E cells.

A subset of cells that expresses CD28 is primarily responsible for the noncytotoxic suppression of HIV replication in CD4⁺ cells [18]. Unfortunately, we have been unable to identify an antibody that recognizes feline CD28. The preliminary observation that CD8α⁺βlo anti-FIV phenotype has lost surface t-selectin and up-regulated CD44 and class II antigen (Gebhard DH, unpublished data) suggests that it is an activation phenotype [28] but does not distinguish between T cytotoxic or T suppressor phenotypes. Reimmann et al. [37] reported that an activated CD8⁺ cell, characterized by increased MHC class II and loss of L-selectin, appears in the lymph nodes of acute-stage simian immunodeficiency virus–infected monkeys [37]. This CD8⁺ activation phenotype did not correlate with CTL activity and the authors speculated it may be a regulatory cell. It is equally possible that this CD8⁺ cell is a noncytotoxic
Figure 7. CD8α⁺βlo cells inhibit FIV production in long-term FIV-infected cats. Peripheral blood mononuclear cells were isolated from cats infected intravenously with FIV NCSU₁ for ≥7 years. Cats had 92.5% (Fred), 93.8% (Dino), and 87.0% (Hoppie) CD8α⁺βlo cells of total monoclonal antibody-positive CD8⁺ cells at time of study. CD8⁺ cells were depleted by immunomagnetic beads and remaining cells cocultured with FCD4E cells. Bars represent SD of mean of triplicate samples. * Unfractionated and CD8⁺ cell-depleted cultures are significantly different (P < .005) by paired t test.

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References

17. Giorgi J, Ho H, Hirji K, et al. CD8\(^+\) lymphocyte activation in human immunodeficiency virus type 1 seroconversion: development of HLA-DR\(^+\) CD8\(^+\) cells is associated with subsequent stable CD4\(^+\) cell levels. J Infect Dis 1994; 170:775–81.