Expansion of CD8αβ cells in cats infected with feline immunodeficiency virus

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Feline immunodeficiency virus (FIV) is a lentivirus which infects domestic cats. The virus has tropisms for feline lymphocyte and macrophage/monocyte lineages, and the pathogenesis of FIV infection is similar to that of human immunodeficiency virus (HIV) infection. Therefore, FIV infection is considered to be a useful animal model for human AIDS (Yamamoto et al., 1988; Ackley et al., 1990b). Although FIV does not utilize the CD4 molecule as a receptor (Norimine et al., 1993), decreased CD4/CD8 ratios have been observed in cats infected with FIV (Ackley et al., 1990b; Lehmann et al., 1992; Willett et al., 1993).

CD8 is a glycoprotein which is expressed on thymocytes and mature T cells, and acts as a co-receptor for the T-cell receptor (TCR) (Swain, 1983). The CD8 glycoprotein is composed of the α- and β-chains in human, mouse, rat and chicken (Golstein et al., 1982; Littman, 1987; Tan et al., 1988; Norment & Littman, 1988; Shiu et al., 1988; Torres-Nagel et al., 1992; Tregaskes et al., 1995) and exists as a heterodimer (αβ) or a homodimer (αα). The former is expressed on CD3+TCRαβ T lymphocytes and the latter is expressed on CD3+TCRγδ T lymphocytes and NK cells in humans (Moebius et al., 1991). Although a population of CD8αβ− cells has been documented in humans and mice (Swain, 1983; Littman, 1987; Terry et al., 1990), no information on this population in cats was reported until our recent study (Shimojima et al., 1997).

To calculate the CD4/CD8 ratio for diagnosis of the degree of immunodeficiency in cats infected with FIV, anti-feline CD8 (fCD8) MAbs FT2 (Klotz & Cooper, 1986), 3.357 (Tomkins et al., 1990) and vpg9 (Willett et al., 1993) have been used so far. Recently, we reported that anti-fCD8 MAb 10C7 (generated in our laboratory) and 3.357, FT2 and vpg9 recognize α-chain, β-chain and αβ-complex epitopes, respectively, and that each MAb displays almost the same reactivities against peripheral blood lymphocytes (PBL) of normal kittens (Shimojima et al., 1997). Although CD8αβ− cells are observed in PBL of healthy humans (Baume et al., 1990), these observations suggested that CD8αβ− cells are not present at significant levels in PBL of normal cats, and that the CD4/CD8 ratio in cats may not differ significantly according to the anti-fCD8 MAb used for detection of fCD8+ lymphocytes.

On the other hand, the rapid expansion of a CD8+ population with weak fluorescence (fCD8low) was documented in FIV-infected cats using FT2 and vpg9 (Lehmann et al., 1992; Hofmann-Lehmann et al., 1995; Willett et al., 1993). We speculated that the fCD8low cells were lymphocytes expressing the CD8β-chain at a low level, although in humans CD8low (or CD8dim) cells are thought to be CD8αα cells expressing the

CD8 lymphocytes have been subdivided into CD8αβ and CD8αα populations in the peripheral blood lymphocytes (PBL) of humans and in several animal species but have not yet been investigated in cats. Feline immunodeficiency virus (FIV) causes progressive immunological disorders similar to human AIDS. In this study, we analysed CD8 cells in PBL of FIV-infected or uninfected cats by two-colour flow cytometric analysis. In specific pathogen-free adult cats, feline CD8αβ high cells were observed but CD8αβ cells were not found in significant numbers. On the other hand, not only CD8αβ high but also CD8αβ and CD8αβ low cell populations were observed in cats chronically infected with FIV. The expansion of the CD8αβlow or CD8αβ subpopulations resulted in the apparent degree of immunodeficiency in cats infected with FIV, anti-fCD8 MAb used. These findings suggest a need to reconsider the CD4/CD8 ratio in studies of FIV infection. Furthermore, we found that the CD8αβ cell population expressed CD5 at a low level.

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CD8α-chain at a low level (Baume et al., 1990). In the present study, to analyse the CD8+ cells in FIV-infected cats in detail, we performed two-colour flow cytometric (FC) analysis with anti-fCD8α, fCD8β, feline CD4 (fCD4) and feline CD5 (fCD5) MAbs on PBL of FIV-infected and uninfected cats.

Feline peripheral blood mononuclear cells (PBMC) were separated on Ficoll–Paque (Pharmacia) from the whole blood of three adult specific pathogen-free (SPF) cats aged 7–9 years (Cats 102, 201 and 202) and three cats infected with each of the FIV Petaluma (Cat 105), TM1 (Cat 103) and TM2 (Cat 104) strains for 8 years (Miyazawa et al., 1989). An aliquot of whole blood was kept for lymphocyte counting by microscopy.

Phycoerythrin (PE)-labelled FT2 (anti-fCD8 MAb) (Klotz & Cooper, 1986), fCD4 (anti-fCD4 MAb) (Ackley et al., 1990a) and f43 (anti-fCD5 MAb) (Ackley & Cooper, 1992) were purchased from Southern Biotechnology Associates (Birmingham, Ala., USA). For labelling of anti-fCD8x MAb with FITC, 2 mg of anti-fCD8x MAb 10C7 was mixed with 0.5 mg FITC–Celite (Sigma) in 0.1 M NaHCO3 buffer (distilled water containing 0.828% NaHCO3, 0.016% Na2CO3, pH 8.5) and left for 10 h at 4°C. The buffer including the MAb conjugated with FITC was then exchanged with PBS by dialysis. The MAb solution was mixed with the same volume of glycerol and used for two-colour FC analysis. MAbs 10C7 and FT2 were used as anti-fCD8x and anti-fCD8β MAb, respectively.

Feline PBMC were washed once with cold sorter buffer (PBS containing 3% foetal calf serum and 0.1% NaN3) and incubated with FITC- or PE-conjugated MAb for 30 min on ice. After washing with the sorter buffer, the stained cells were analysed on a FACSscan (Becton Dickinson). We performed the analysis four or more times, and similar patterns of subpopulations were always observed. Representative results of two-colour FC are shown in Fig. 1. Numbers of cell subpopulations were calculated from both absolute numbers of lymphocytes and percentage FC analysis (Fig. 2a). Ratios of fCD4/fCD8x and fCD4/fCD8β are also shown in Fig. 2(b).

Anti-fCD8x MAb (10C7) and anti-fCD8β MAb (FT2) recognized virtually identical populations in the PBL of SPF adult cats [Fig. 1 (202-1), Fig. 2a]. The same reactivities of a panel of anti-fCD8 MAbs were also observed in the PBL of 1-month-old kittens (Shimojima et al., 1997), suggesting that fCD8αβ− cells might not be present in significant numbers in PBL of SPF cats at any stage. On the other hand, in the PBL of FIV-infected cats, we found increases in the absolute number and percentages of a fCD8αβ− cell population as well as a fCD8αβlow cell population, which could not be observed at significant levels in uninfected cats [Fig. 1 (103-1), Fig. 2a]. From these results, we conclude that the fCD8αβlow cell population reported by Lehmann et al. (1992) and Willett et al. (1993) is fCD8αββlow cells because they used FT2 (anti-fCD8β) and vpg9 (anti-fCD8β) MAbs for determination of the cell population. The population might be distinctly different from NK cells with CD8αlow lacking β-chain observed in humans (Baume et al., 1990). The fluorescence intensity of the α-chain of fCD8αββlow cells was similar to that of fCD8αββhigh cells, suggesting that fCD8αββlow cells express the α-chain at the same level as fCD8αββhigh cells and β-chain at a low level. Such a cell population has not been reported in mammalian or avian species, and its origin and role are unclear. In view of their cell surface phenotype and fluorescence intensity, the fCD8αβ− population in FIV-infected cats; therefore the screen was divided into six areas.

![Fig. 1.](image-url) Representative results of the two-colour FC analysis of IPBMC from FIV-infected (202) and uninfected (103) SPF cats. The IPBMC were stained with pairs of MAbs as indicated. For detection of fCD8α, fCD8β, fCD4 and fCD5 molecules, anti-fCD8α MAb (10C7) conjugated with FITC, anti-fCD8β MAb (FT2) conjugated with PE, anti-fCD4 MAb (fCD4) conjugated with PE, and anti-fCD5 MAb (f43) conjugated with PE were used, respectively. The number in the corner of each panel indicates the percentage of cells in each area. In the case of CD8α−CD8β− analysis, three subpopulations were observed in the CD8α− population in FIV-infected cats; therefore the screen was divided into six areas.


**Fig. 2.** (a) Absolute cell numbers of each subpopulation of FIV-uninfected cats (102, 201 and 202) and infected (103 and 104) SPF cats. CD8β+ indicates a lymphocyte population reactive to FT2 MAb. (b) CD4/CD8 ratios of FIV-uninfected (102, 201 and 202) and infected (103, 104 and 105) cats.

Cell population may be analogous to non-MHC-restricted T lymphocytes found in human blood (Baume et al., 1990; Moebius et al., 1991).

Expansion of the fCD8αβ− cells in FIV-infected cats resulted in a significant difference of fCD4/fCD8 ratio depending on the anti-fCD8 MAb used (Fig. 2b). The fCD4/fCD8 ratios calculated on the basis of β-specific MAb reactivity appeared higher than with the α-specific MAb. In the study of human AIDS, OKT8 recognizing the α-chain of CD8 (Shiu et al., 1988) is generally used to detect CD8, while the FT2 MAb which recognizes the β-chain of fCD8 (Klotz & Cooper, 1986) has been widely used for examination of the fCD8+ cell population in FIV-infected cats. Anti-fCD8β MAb can detect expansion of fCD8αβlow cells but will not reveal fCD8αβ− cells. On the other hand, anti-fCD8α MAb will detect fCD8 populations which include βlow and β− cells without distinguishing between them. To study the functions and roles of the fCD8 subpopulations in FIV-infected cats, it will be necessary to use α-specific MAb as well as β-specific MAb, and a re-evaluation of CD4/CD8 ratios in FIV-infected cats might be needed.

To confirm the expansion of the subpopulations after FIV infection, we did a further analysis with an additional four SPF cats. Two 5-5-month-old (Cats 304 and 305) and two 8-month-old (Cats 302 and 303) female SPF cats purchased from Harlan Sprague–Dawley were inoculated with FIV (TM2 strain)-infected MYA-1 cells (Miyazawa et al., 1989a, b) via the vaginal mucosa. Blood samples were collected at 16 weeks post-inoculation (p.i.) (in Cats 302 and 303) and 25 weeks p.i. (in Cats 304 and 305), and PBMC were separated, and then analysed by FC. The appearance of the fCD8αβ−βlow or fCD8αβ− subpopulations was confirmed even at the early stage of infection (Fig. 3).

In this study, the fCD8αβCD5low population was also observed in FIV-infected cats (Cats 103 and 104) [Fig. 1 (103-3), Fig. 2a] including Cat 105 and Cats 302–305 (data not shown), while such a population was not observed at significant levels in uninfected cats [Fig. 1 (202-3), Fig. 2a]. The PE fluorescence-intensity of the fCD8αβCD5low population was not significantly altered by addition of PE-labelled-anti-fCD8β MAb [Fig. 1 (103-4)], and the number of the fCD5low cells was similar to that of fCD8αβ− cells (Fig. 2a). These data indicate that the phenotype of the cells might be CD5lowCD8αβ−. It remains unclear whether the population corresponds to the CD3+CD8+CD5− population which is expanded in HIV-infected patients (Indraccolo et al., 1995), since anti-feline CD3 MAbs for FC analysis are not available at present. Further studies on the fCD8αβCD5low cells are also needed to understand the role of this subpopulation in FIV-infected cats.

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References


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