Short Communication

Downmodulation of CD3ε expression in CD8α+β− T cells of feline immunodeficiency virus-infected cats

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Feline immunodeficiency virus (FIV) infection in cats is associated with an increase of feline CD (fCD)8α+β− and fCD8α+βlow cells in peripheral blood. To investigate these cells in more detail, an anti-fCD3ε mAb, termed NZM1, was generated, which recognizes the extracellular epitope of the fCD3ε molecule. The anti-fCD3ε mAb proved to be more suitable for identifying feline T cells than the anti-fCD5 one, which has been used as a pan-T-cell reagent in cats, because of the presence of fCD5+fCD3ε− cells among lymphocytes. Although the fCD8α+β− and fCD8α+βlow cells in the FIV-infected cats expressed fCD3ε, a subset of fCD8α+β− cells expressed fCD3ε antigen at a lower level than the T cells whose phenotype was fCD4+, or fCD8α+βlow. The lower expression of fCD3ε may be associated with the immune status of fCD8α+β− T cells.

CD8+ T cells play potential roles in the immunopathogenesis of human immunodeficiency virus type 1 (HIV-1) infection (Yang & Walker, 1997). The CD8 antigen consists of two polypeptides, CD8α and CD8β, and exists as a heterodimer (CD8αβ) or a homodimer (CD8αα). In humans, T-cell receptor (TCR) αβ T cells express the CD8αβ heterodimer, and TCRγδ T cells and natural killer cells express the CD8αα homodimer (Moebius et al., 1991). Feline immunodeficiency virus (FIV) infection in cats has been studied extensively as an animal model for the persistent infections and pathogenesis caused by HIV (for a review see Miyazawa, 2002). Previously, we found that feline CD (fCD)8α+β− and fCD8α+βlow cells increased in number in the peripheral blood of FIV-infected cats (Shimojima et al., 1998a). These subsets were reported to play roles in the suppression of FIV replication (Bucci et al., 1998; Flynn et al., 2002; Gebhard et al., 1999; Shimojima et al., 2004). The induction of similar subpopulations was also confirmed in human diseases, such as HIV infection (Schmitz et al., 1998). However, it remains unknown whether the fCD8α+β− and fCD8α+βlow cells are T cells or natural killer cells. The phenotypic characterization of fCD8α+β− and fCD8α+βlow cells in FIV-infected cats is difficult due to a lack of monoclonal antibodies (mAbs) against appropriate surface markers.

Cells of the T-cell lineage bear a TCR–CD3 complex consisting of variable αβ or γδ TCR chains associated with invariant CD3 chains of γ, δ, ε, and ζ (Ashwell & Klausner, 1990). The CD3ε chain appears to be the most immunogenic and exposed part of CD3, as anti-human CD3 mAbs are predominantly directed to epitopes of the CD3ε subunit (Transy et al., 1989). Only completely assembled TCR–CD3 complex can be expressed on the T-cell surface (Clevers et al., 1988). Therefore, mAbs for CD3ε have exquisite specificity for T cells and are widely used to identify T cells.
in both humans (Reinherz et al., 1979) and mice (Leo et al., 1987). To investigate feline T cells, Joling et al. (1996) reported that an anti-human CD3ε polyclonal antibody, prepared from rabbits immunized with peptides of the cytoplasmic domain of human CD3ε, cross-reacted with feline CD3ε and could be used for immunohistochemical studies in cats. However, this antibody was inconvenient as the permeabilization of cells is necessary for flow cyto-
metric analysis. Instead of a specific anti-fCD3 mAb, f43 mAb, which recognizes the feline homologue of the CD5 antigen, has been used as a pan-T-cell reagent in cats (Ackley & Cooper, 1992). However, the CD5 molecule is inappropriate for the detection of feline T cells. In order to solve this problem, we prepared a mAb termed NZM1 (IgG3) was selected based on the reactivity with a T-lymphoblastoid cell line, MYA-1 cells (Miyazawa et al., 1987; Tsoukas et al., 1985; Yang et al., 1996). Feline PBMCs (2 x 10⁶) separated from heparinized whole blood of a specific-pathogen-free (SPF) cat were suspended in 100 μl RPMI 1640 medium containing fetal calf serum (10 %, v/v) and antibiotics, and plated in a well of a 96-well flat-bottomed microculture plate. The PBMCs were cultured in the presence of the anti-fCD4 mAb [4D9 (IgG1); Shimojima et al., 1997], anti-fCD8a [12A3 (IgG2a); Shimojima et al., 1998b] or NZM1 (final dilution, ascites 1:10⁴, 1:10³ or 1:10²) for 72 h at 37°C in a humidified atmosphere of 5 % CO₂ in air. The proliferation of PBMCs was measured by MTT assay (Mosmann, 1983). The cells proliferated to a greater extent when cultured with NZM1 than with 4D9 or 12A3 (P<0.005, n=3; data not shown). We considered that NZM1 recognizes the extracellular epitope of fCD3ε, as it could stain feline PBMCs without permeabilization in the immunofluorescence analysis and induce the proliferation of feline PBMCs in the co-cultivation experiments.

Two cats infected with each of the FIV TM1 (cat 103) and TM2 (cat 104) strains for 11 years (Miyazawa et al., 1989a) and one infected with the Petaluma strain for 2 years (cat 115) were used in the flow cytometric analysis. Three adult SPF cats aged 8–10 years (cats 102, 201 and 202) were used as uninfected controls. All cats were clinically healthy. PBMCs were suspended in a sorter buffer (PBS containing 3 % fetal calf serum and 0.05 % sodium azide) and centrifuged at 800 r.p.m. to remove platelets. The mAb NZM1 was labelled with FITC (fCD3ε–FITC) according to a standard procedure. PBMCs were washed twice in the cold sorter buffer and incubated with fCD3ε–FITC. After washing with the sorter buffer, stained cells were analysed after gating for lymphocytes based on light (forward and side) scatter using a flow cytometer FACScan with CELLQUEST software (Becton Dickinson). The different subpopulations were expressed as percentages of the total lymphocyte population. The uninfected and FIV-infected groups gave distinctive patterns of fCD3ε expression, and representative results are shown in Fig. 2. In FIV-uninfected SPF cats, the fCD3ε molecule was expressed on 57.2 ± 9.5 % (n=3) of

**Fig. 1.** Immunoblotting analysis of S/9 cells (lanes 1–3 and 6–8), MYA-1 cells (lanes 4 and 9) and feline PBMCs (lanes 5 and 10) using anti-human CD3ε polyclonal antibody (lanes 1–5) and NZM1 mAb (lanes 6–10). Positive reactions were visualized by 3,3′-diaminobenzidine tetrahydrochloride staining. The S/9 cells were mock-infected (lanes 1 and 6) or infected with the control baculovirus (lanes 2 and 7) or rAcfCD3ε (lanes 3 and 8). Specific bands were observed in lanes 3–5 and 8–10.
Characterization of feline CD8a+β− and CD8a+βlow cells

peripheral lymphocytes (Fig. 2a). On the other hand, two subsets of CD3+ cells, CD3high (33.1 ± 16.5 %, n = 3) and CD3low (20.7 ± 9.3 %, n = 3), were detected in the FIV-infected cats (Fig. 2c). As the CD5 antigen has been considered a pan-T-cell molecule in cats, PBMCs were labelled with CD3–FITC and phycoerythrin (PE)-conjugated anti-CD5 mAb (CD5–PE), f43 (Ackley & Cooper, 1992) and analysed by flow cytometry (Fig. 2b, d). Although most of the CD5 cells expressed the CD3 molecule, there was a substantial number of CD5low CD3low cells in FIV-uninfected SPF cats (2.0 ± 1.7 %, n = 3; Fig. 2b). So anti-CD5 mAb appears to be unsuitable for the detection of feline T cells. The expression of CD5 antigen on feline B cells has not been characterized in detail, and it is unknown whether this subset corresponds to CD5+B cells in humans and mice. It should also be noted that the CD3low population consisted of CD3high and CD3low subsets (Fig. 2d), which indicates that CD8+β− cells in FIV-infected cats consist of CD3high and CD3low subsets (Shimojima et al., 1998a; Stievano et al., 2003).

Next the PBMCs were stained with mAb fCD3–FITC and either fCD4–PE (Fel7; Ackley et al., 1990), fCD8β–PE (FT2; Klotz & Cooper, 1986), fCD8a (2D7; Shimojima et al., 1998b), or the mixture of fCD4–PE, fCD8a and fCD8β–PE. A secondary rat anti-mouse IgG2a antibody conjugated with PE (Zymed Laboratories) was used for the detection of fCD8a. The uninfected and FIV-infected groups gave distinctive patterns, and representative results are shown in Fig. 3. Most of the fCD4+ and fCD8+ cells were fCD3−. The fCD3− cell population consisted of fCD4+ (46.3 ± 2.4 %; Fig. 3a), fCD8a+ (41.9 ± 2.3 %; Fig. 3b) and fCD4− fCD8a−β− (9.3 ± 0.6 %; Fig. 3d) cells in the SPF cats (n = 3). Most of the fCD3low cells in the FIV-infected cats were fCD8low fCD4− fCD8a+β− (Fig. 3d–g). In addition, fCD8low cells whose population expanded in FIV-infected cats also expressed fCD3− (Fig. 3c, g).

The fCD8a+β− and fCD8a+βlow cells in the FIV-infected cats expressed fCD3−, hence these subsets are T cells. It is still unknown at present whether fCD8a+β−, fCD8b+βlow and fCD3− fCD4+ fCD8a+β− cells are γδ T cells, as no reagent specific for the feline TCR γ− or δ-chain is available. We also found a lower level of expression of the fCD8a molecule in fCD8a+β− subsets. A decreased expression of CD8a is reported in CD3+ cells but not natural killer cells in HIV-infected individuals (Ginaldi et al., 1997). Down-regulation of fCD8 expression may contribute to the progressive reduction of fCD8+ cell function in FIV-infected cats. Several factors may be involved in the change of fCD3 expression in FIV infection. In general, the CD3low T cell is a recently antigen-activated or memory cell. It is reported that both activated and non-activated T cells from HIV-positive patients express less CD3 than those from control subjects (Ginaldi et al., 1997). As CD3+ plays an important role in signalling of TCR/CD3, fCD3low cells might raise the activation threshold and contribute to the lack of effective immune surveillance. There is a continuous loss of naïve CD4 and CD8 T cells and expansion of memory cells in HIV-infected patients (Bass et al., 1992). As the majority of fCD8a+β− cells show an increase in fCD11a expression, one of the activation antigens (Shimojima et al., 2003) and CD8a+β− memory T cells descend directly from clonally expanded CD8a+β− cells (Konno et al., 2002), we speculate that fCD3low fCD8a+β− T cells consist of activated memory subsets. Hohdatsu et al. (2003) reported controversial anti-FIV activities of fCD8a+β− and fCD8b+βlow subsets. Not all fCD8a+β− and fCD8b+βlow cells, but some with enough fCD3 expression, may have strong anti-FIV activity.

Trimble & Lieberman (1998) reported the expansion of CD3low subsets in a substantial fraction of CD8+ T cells in HIV-infected patients. They classified the CD8+ cells into the subpopulations CD8+ CD3− and CD8+ CD3+. They did not mention the fluorescent intensity of the CD3e molecule on CD3+ cells, and concluded that the downregulation of CD3e expression is independent of other TCR/CD3 components. A decrease in CD3e mRNA levels was also reported in T cells from AIDS patients (Geertsma et al., 1999), but that of CD3e mRNA levels has not yet been discussed. Although downregulation of CD3
expression on CD4⁺ and CD8⁺ cells is reported in HIV-infected patients, its relationship with CD3e expression is unclear (Ginaldi et al., 1997). In the fCD3 complex, fCD3e is the only molecule whose cDNA has been identified, and NZM1 is the first mAb specific to the fCD3 component. Therefore it is not known at present whether the fCD3e downregulation involves a decrease of other feline TCR/CD3 components, including fCD3f. If the downregulation of fCD3e in the fCD8⁺ cells of FIV-infected cats correlates with disease progression, as does that of CD3f in HIV infection (Geertsma et al., 1999), the measurement of fCD3e expression may contribute to our understanding of the immune status of FIV-infected cats.

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References


Konn, A., Okada, K., Mizuno, K. & 9 other authors (2002). CD8αβ memory effector T cells descend directly from clonally expanded CD8αβhigh TCRαβ+ T cells in vivo. Blood 100, 4090–4097.


