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 fCD5ε+CD3ε− 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 cytometric 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 that detects the fCD3ε antigen in immunoblotting and flow cytometric analyses, and characterized the fCD8α+β− and fCD8α+βlow cells in FIV-infected cats.

Hybridomas were generated from BALB/c mice immunized with insect cells (Sf9 cells) infected with the recombinant baculovirus rAcfCD3ε, which carries cDNA encoding the fCD3ε molecule (Nishimura et al., 1998). A positive hybridoma designated NZM1 (IgG3) was selected based on the reactivity with a T-lymphoblastoid cell line, MYA-1 cells (Miyazawa et al., 1989b), by an indirect immunofluorescence assay using a fluorescein isothiocyanate (FITC)-conjugated secondary antibody. The specificity of NZM1 was confirmed by the immunoblotting analysis using Sf9 cells infected with rAcfCD3ε and feline peripheral blood mononuclear cells (PBMCs) as antigens (Fig. 1). As a control, a rabbit polyclonal antibody against the cytoplasmic region of human CD3ε (Dako A/S) was used. Secondary antibodies conjugated with horseradish peroxidase were used to detect positive signals as described previously (Miyazawa et al., 1989a). NZM1 recognized several bands of about 25 kDa in Sf9 cells infected with rAcfCD3ε (Fig. 1, lane 8) but not in mock-infected cells (Fig. 1, lane 6) or cells infected with the control baculovirus (Fig. 1, lane 7). NZM1 was confirmed to react with a 25 kDa molecule of MYA-1 cells (Fig. 1, lane 9) and feline PBMCs (Fig. 1, lane 10), which was identical to the molecule recognized by the anti-human CD3ε polyclonal antibody (Fig. 1, lanes 1–5). These findings indicate that the mAb NZM1 is directed against the fCD3ε molecule.

Next, we investigated whether the engagement of fCD3ε with NZM1 also induced T-cell proliferation as demonstrated with anti-CD3 mAbs of other species (Leo et al., 1987; Tsoukas et al., 1985; Yang et al., 1996). Feline PBMCs (2 × 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-CD4 mAb [4D9 (IgG1); Shimojima et al., 1997], anti-fCD8α [12A3 (IgG2a); Shimojima et al., 1998b] or NZM1 (final dilution, ascites 1:10³, 1:10⁴ or 1:10⁵) 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 sort 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 sort buffer and incubated with fCD3ε–FITC. After washing with the sort buffer, stained cells were analysed after gating for lymphocytes based on light (forward and side) scatters 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 Sf9 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 Sf9 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.](image-url)
Characterization of feline CD8α+β− and CD8α+βlow cells

so anti-fCD5 mAb appears to be unsuitable for the detection of feline T cells. The expression of fCD5 antigen has been considered a pan-T-cell molecule in cats, and it was reported that both activated and non-activated T cells from HIV-positive patients express less CD3 than those from control subjects (Ginaldi et al., 1990), but that of CD3 expression in FIV infection. In general, the CD3low molecule of fCD8α+β− cells but not natural killer cells in HIV-infected individuals (Ginaldi et al., 1997). Down-regulation of CD8 expression may contribute to the progressive reduction of CD8+ cell function in FIV-infected cats. Several factors may be involved in the change of fCD3c 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 CD3c 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). The majority of fCD8α+β− cells show an increase in fCD11a expression, one of the activation antigens (Shimojima et al., 2003) and CD8αβ− memory T cells descend directly from clonally expanded CD8αβ+ T cells (Konno et al., 2002), we speculate that fCD3clow fCD8αβ− T cells consist of activated memory subsets. Hohdatsu et al. (2003) reported controversial anti-FIV activities of fCD8αβ− and fCD8αβ+ low subsets. Not all fCD8αβ− and fCD8αβ+ low cells, but some with enough fCD3c expression, may have strong anti-FIV activity.

Trimble & Lieberman (1998) reported the expansion of CD3c− subsets in a substantial fraction of CD8+ T cells in HIV-infected patients. They classified the CD8+ cells into the subpopulations CD8+CD3c− and CD8+CD3c+. They did not mention the fluorescent intensity of the CD3c molecule on CD3+ cells, and concluded that the downregulation of CD3c expression is independent of other TCR/CD3 components. A decrease in CD3c mRNA levels was also reported in T cells from AIDS patients (Geertsma et al., 1999), but that of CD3c mRNA levels has not yet been discussed. Although downregulation of CD3

Next the PBMCs were stained with mAb fCD3e–FITC and either fCD4–PE (Fe7; Ackley et al., 1990), fCD8β–PE (FT2; Klotz & Cooper, 1986), fCD8α (2D7; Shimojima et al., 1998b), or the mixture of fCD4–PE, fCD8α and fCD8β–PE. A secondary rat anti-mouse IgG2a antibody conjugated with PE (Zymed Laboratories) was used for the detection of fCD8α. The unidentified and FIV-infected groups gave distinctive patterns, and representative results are shown in Fig. 3. Most of the fCD4+ and fCD8− cells were fCD3c+. The fCD3c+ cell population consisted of fCD4+ (46.3±2.4%; Fig. 3a), fCD8α− (41.9±2.3%; Fig. 3b) and fCD4− fCD8α+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− fCD8α+β− (Fig. 3d–g). In addition, fCD8βlow cells whose population expanded in FIV-infected cats also expressed fCD3c (Fig. 3c, g).

The fCD8α+β− and fCD8α+βlow cells in the FIV-infected cats expressed fCD3c, hence these subsets are T cells. It is still unknown at present whether fCD8α+β−, fCD8α+βlow and fCD3c+ fCD4− fCD8α+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 fCD8α molecule in fCD8α+β− subsets. A decreased expression of CD8α is reported in CD3+ cells but not natural killer cells in HIV-infected individuals (Ginaldi et al., 1997). Down-regulation of CD8 expression may contribute to the progressive reduction of CD8+ cell function in FIV-infected cats. Several factors may be involved in the change of fCD3c 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 CD3c 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 fCD8α+β− cells show an increase in fCD11a expression, one of the activation antigens (Shimojima et al., 2003) and CD8αβ− memory T cells descend directly from clonally expanded CD8αβ+ T cells (Konno et al., 2002), we speculate that fCD3clow fCD8αβ− T cells consist of activated memory subsets. Hohdatsu et al. (2003) reported controversial anti-FIV activities of fCD8αβ− and fCD8αβ+ low subsets. Not all fCD8αβ− and fCD8αβ+ low cells, but some with enough fCD3c expression, may have strong anti-FIV activity.

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expression on CD4$^+$ and CD8$^+$ cells is reported in HIV-infected patients, its relationship with CD3$^c$ expression is unclear (Ginaldi et al., 1997). In the ICD3 complex, ICD3e is the only molecule whose cDNA has been identified, and NZM1 is the first mAb specific to the ICD3 component. Therefore it is not known at present whether the ICD3e downregulation involves a decrease of other feline TCR/CD3 components, including ICD3f. If the downregulation of ICD3e in the ICD8$^+$ cells of FIV-infected cats correlates with disease progression, as does that of CD3f in HIV infection (Geertsma et al., 1999), the measurement of ICD3e expression may contribute to our understanding of the immune status of FIV-infected cats.

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**References**


