Altered Surface Antigen Expression on Peripheral Blood Mononuclear Cells in Cats Infected with Feline Immunodeficiency Virus

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ABSTRACT. Expression of CD4, CD8, IL-2 receptor α chain (IL-2Rα), and MHC class II (MHC-II) on peripheral blood mononuclear cells were examined in cats infected with feline immunodeficiency virus (FIV). CD4/CD8 T cell ratio in FIV-infected cats was slightly decreased, as compared with that in specific-pathogen-free (SPF) cats. However, there was no statistical differences between them. The number of circulating IL-2Ra⁺ cells in FIV-infected cats was higher than that in healthy cats, whereas induction of IL-2Ra expression by concanavalin A (Con A) stimulation was depressed in FIV-infected cats. By using two-color cytofluorometry, Con A-induced enhancement of IL-2Ra expression was found to be reduced in both CD4⁺ and CD8⁺ populations in PBMC from FIV-infected cats. The circulating MHC-II⁺ cells were also increased in FIV-infected cats. Furthermore, the induction of IL-2Ra expression on PBMC after Con A-stimulation significantly depressed by FIV inoculation in vitro. These results suggest that FIV activates PBMC in vivo via direct and/or indirect mechanisms, leading to the unresponsive state of T cells to further stimuli in vitro.—KEY WORDS: CD4, CD8, FIV, IL-2Ra, MHC class II.

Feline immunodeficiency virus (FIV) was first isolated in 1987 from cats suspected to have immunodeficiency disease [16]. FIV, which belongs to a subfamily lentivirinae in retroviridae, resembles human immunodeficiency virus (HIV) in morphology, molecular and biochemical characteristics and immunosuppressive potentials [13, 16, 21, 27]. Therefore, FIV infection of cats may serve as an animal model for human acquired immunodeficiency syndrome (AIDS).

Clinical signs commonly seen in FIV-infected cats include stomatitis/gingivitis, rhinitis, anemia, emaciation and opportunistic infections [6, 16]. Studies of experimental infection of specific-pathogen-free (SPF) cats showed that FIV induces fever, lymphadenopathy and decrease of CD4⁺ lymphocytes in number [23, 27].

In HIV infection, the progressive depletion of CD4⁺ T cell subset appears to be very important for the pathogenesis of AIDS [7]. However, there has been little information on cytopathogenic and immunopathogenic changes in FIV infection. A recent report indicated that mitogen-induced lymphocyte blastogenesis is suppressed in FIV-infected cats [22]. The poor T cell proliferative responses have been also observed in HIV infection [4, 10], and both IL-2 secretion and expression of IL-2 receptor α (IL-2Ra) were shown to be impaired in these AIDS patients [17, 24].

In the present study, we investigated the expression of CD4, CD8, IL-2Ra and MHC class II (MHC-II) on PBMC obtained from cats naturally infected with FIV to know the mechanism of impairment of lymphocyte functions in FIV-infected cats.

MATERIALS AND METHODS

Cats: Six cats (4–10 years of age) positive for FIV antibody, admitted to the Veterinary Medical Center, the University of Tokyo, were used as naturally infected animals. These cats were negative for FeLV antigen. All these cats showed clinical signs of stomatitis/gingivitis or chronic upper respiratory disease. These cats were categorized to be in the clinical stage of AIDS-related complex (ARC) by the criteria modified from those in HIV infection [5]. Six SPF cats (3–6 years of age) were used as uninfected controls.

Antibodies: Fel 7 [1] and FT2 [8] were mouse monoclonal antibodies (mAbs) to feline CD4 and CD8, respectively, which were kindly provided by Dr. Max D. Cooper, Howard Hughes Medical Institute, University of Alabama at Birmingham.

9F23, which was a mouse mAb to feline IL-2Ra, was produced in our laboratory [12]. ISCR3, which was a generous gift of Dr. N. Shinohara (Lab. Cell. Immunol., Mitsubishi Kasei Institute of Life Science, Tokyo, Japan), was directed to mouse I-E and cross-reacted with feline Ia antigens [25]. Fel 7, FT2
and ISCR3 were used as an undiluted hybridoma supernatant. 9F23 was obtained from the ascites of pristane-pretreated mice growing the hybridoma as a tumor, and was purified by protein A-Sepharose affinity chromatography. Biotinylation of 9F23 was performed by a standard protocol.

Cells and cell culture: Feline PBMC were separated from heparinized peripheral blood by centrifugation on a Lymphoprep gradient (SG: 1.077, NYCOMED, Norway) at 400 × g for 30 min. PBMC at the interface were harvested and washed three times with PBS. The cell concentration was adjusted to 2×10⁶ cells/ml and then cultured in RPMI 1640 containing 10% heat-inactivated FCS, penicillin (50 U/ml), streptomycin (50 μg/ml) and L-glutamine (2 mM).

Immunofluorescence assays for surface antigen expression: Assays for expression of surface antigens were performed by indirect immunofluorescence for both single and two-color analysis. PBMC were incubated first with mouse mAbs at 4°C for 30 min and washed with PBS, then incubated with FITC-conjugated goat antibody to mouse IgG (Cappel Products, U.S.A.). In two-color immunofluorescence experiments, the stained cells were reacted with a second biotinylated antibody at 4°C for 30 min followed by PE-conjugated streptavidin (Tago, INC., U.S.A.). Normal serum was added before reacting with secondary antibodies for blocking residual reactants. These samples were analyzed by CYTO-ACE cytofluorometer (Japan Spectroscopic Co., Tokyo, Japan).

Treatment of Con A-stimulated PBMC with FIV: FIV-Petaluma virion from the supernatant of FIV-CRFK cells [16, 27] were concentrated and purified by ultracentrifugation as previously described [16]. PBMC (2×10⁶ cells/ml) from SPF cats were cultured with concanavalin A (Con A, Sigma, U.S.A., 15 μg/ml) for 72 hr, and then these cells were washed and resuspended in RPMI 1640 containing recombinant human IL-2 (100 U/ml, Shionogi Pharm. Co., Ltd., Osaka, Japan), polybrene (2 μg/ml) and FIV virion (reverse transcriptase activity, 50,000 cpn/ml). After incubation for 72 hr, these cells were analyzed for the expression of CD4, CD8, IL-2Rα and MHC-II.

RESULTS

CD4/CD8 T cell ratio: The relative proportions of CD4⁺ and CD8⁺ subsets in T lymphocytes were examined by cytofluorometry in SPF and ARC cats. As shown in Table 1, the CD4/CD8 ratio in FIV-infected cats (1.60±0.17) was slightly decreased, as compared with that in SPF cats (2.03±0.46). However, there was no statistical differences between them (P>0.05).

Table 1. CD4/CD8 T cell ratio of PBMC in SPF and FIV-infected cats

<table>
<thead>
<tr>
<th>Cats</th>
<th>CD4/CD8⁺⁺⁺ (mean±SD)</th>
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<tbody>
<tr>
<td>SPF</td>
<td>No.1: 3.00</td>
</tr>
<tr>
<td></td>
<td>No.2: 1.62</td>
</tr>
<tr>
<td></td>
<td>No.3: 2.12</td>
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<tr>
<td></td>
<td>No.4: 1.86</td>
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<tr>
<td></td>
<td>No.5: 1.82</td>
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<tr>
<td></td>
<td>No.6: 1.77</td>
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<tr>
<td></td>
<td>(2.03±0.46)</td>
</tr>
<tr>
<td>FIV-infected</td>
<td>No.1: 1.38</td>
</tr>
<tr>
<td></td>
<td>No.2: 1.58</td>
</tr>
<tr>
<td></td>
<td>No.3: 1.67</td>
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<tr>
<td></td>
<td>No.4: 1.90</td>
</tr>
<tr>
<td></td>
<td>No.5: 1.63</td>
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<tr>
<td></td>
<td>No.6: 1.45</td>
</tr>
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<td>(1.60±0.17)</td>
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</table>

a) The percentages of PBMC expressing CD4 and CD8 were determined by immunofluorescence.

IL-2Rα expression on PBMC in FIV-infected cats: Freshly-isolated and Con A-stimulated PBMC were analyzed for IL-2Rα expression by using 9F23 mAb. The percentage of circulating IL-2Rα⁺⁺ cells in SPF cats ranged between 8 to 20% (Mean±SD, 13.8±4.4), whereas that in FIV-infected cats was significantly increased with of 63.2±19.5% (P<0.01) (Fig. 1). To examine the ability of PBMC to express IL-2Rα after in vitro mitogen stimulation, PBMC was incubated with Con A (15 μg/ml) for 3 days before the phenotypic analysis. The mean±SD of IL-2Rα⁺⁺ cells in Con A-stimulated PBMC of SPF and FIV-infected cats were 90.3±6.4% and 71.5±11.5%, respectively. These findings indicate that the enhancement of IL-2Rα expression by in vitro stimulation is significantly decreased (P<0.01) in FIV-infected cats.

Furthermore, we examined the IL-2Rα expression on T cell subpopulations after Con A-stimulation by two-color cytofluorometry. A representative result was shown in Fig. 2. The percentages of IL-2Rα⁺⁺⁺ cells in both CD4⁺⁺⁺ and CD8⁺⁺⁺ cells were decreased in FIV-infected cats (48.1% and 64.3%), as compared with that in SPF cats (96.1% and 90.1%).

MHC-II expression on PBMC of FIV-infected
ALTERED ANTIGEN EXPRESSION BY FIV

Fig. 1. IL-2Ra expression on PBMC in SPF and FIV-infected cats. PBMC were stained with 9F23 mAb followed by FITC-conjugated goat antibody to mouse IgG. Data represent percentage of IL-2Ra<sup>+</sup> cells before and after cultivation with 15 μg/ml Con A for 72 hr.

Fig. 2. Cyttofluorometric analysis of CD4, CD8 and IL-2Ra expression on PBMC in SPF and FIV-infected cats. PBMC were stained with Fcε 7 or FT2 mAb followed by FITC-conjugated goat antibody to mouse IgG, then stained cells were reacted with biotinylated 9F23 mAb followed by PE-conjugated streptavidin.

Fig. 3. MHC class II expression on PBMC in SPF and FIV-infected cats. Data represent percentage of MHC class II<sup>+</sup> cells before and after cultivation with Con A for 72 hr.

cats: MHC-II expression was examined in freshly-isolated and Con A-stimulated PBMC by using mAb against murine I-E. As shown in Fig. 3. a significantly higher percentage of circulating MHC-II<sup>+</sup> cells was observed in FIV-infected cats (Mean±SD, 78.8±9.4%), as compared with that in SPF cats (Mean±SD, 52.3±12.1%) (P<0.05). This observation is consistent with the increased expression of IL-2Ra. In Con A-stimulated PBMC, the percentage of MHC-II<sup>+</sup> cells did not significantly differ between SPF and FIV-infected cats.

Effect of FIV virion on surface antigen expression in Con A-stimulated normal PBMC: To examine whether FIV could directly influence the expression of surface antigens in vitro, PBMC from SPF cats stimulated with Con A for 3 days were exposed to FIV and monitored for the expression of CD4, CD8, IL-2Ra and MHC-II after cultivation for 3 days in the presence of IL-2. As shown in Fig. 4, the expression of CD4, CD8 and MHC-II did not differ between control and FIV-inoculated PBMC. However, the expression of IL-2Ra was significantly suppressed by FIV inoculation; mean fluorescence intensities in control and FIV-inoculated cells were 86.6 and 67.3, respectively. FIV appeared to depress the induction of IL-2Ra expression on those cultured lymphocyte after mitogen-stimulation in a dose-dependent manner (data not shown).
HIV infects helper/inducer T cells through its receptor CD4, and causes AIDS as the result of a progressive depletion of CD4+ T cells. Although the major target of HIV is CD4+ T cells, profound immunologic defects in other cell lineages (B cells, CD8+ T cells and monocytes) have also been described in AIDS patients [13, 15, 20].

FIV has been shown to be an etiologic agent of feline AIDS, however, the mechanism of its pathogenesis is still unknown. Recently, the depressed proliferative responses of PBMC to mitogens, as observed in HIV infection, have also been reported in FIV-infected cats [22]. To determine the cause of the impaired proliferative response of PBMC in FIV infection, we investigated the surface antigen expression on PBMC in FIV-infected cats by using mAbs directed to CD4, CD8, IL-2Rα and MHC-II.

In the present study, an apparent decrease of CD4/CD8 T cell ratio was not observed in ARC cats. Ackley et al. reported that the cats experimentally infected with FIV had depressed CD4/CD8 ratio resulting from a decrease in circulating CD4+ cells [2]. The inconsistency of our results concerning CD4/CD8 ratio in FIV-infected cats from those reported by Ackley et al. may be attributed to the difference of clinical stages of the infected cats used. In our preliminary study, the ability of IL-2 production in Con A-stimulated PBMC was not significantly impaired in ARC cats (data not shown), suggesting that the functions of CD4+ cells, which are the main source of IL-2, are not impaired at the clinical stage of ARC in FIV infection.

Activation and proliferation of T cells are induced by secretion of IL-2, expression of IL-2R and signal transduction after its ligand-receptor binding. The number of circulating PBMC which expressed IL-2Rα was increased in FIV-infected cats. However, the induction of IL-2Rα expression on PBMC after Con A-stimulation was significantly depressed in FIV-infected cats. Depressed IL-2Rα expression on PHA-stimulated PBMC has been also reported at all stages of HIV infection [17, 24]. It is likely that FIV activate T cells in vivo through direct infection and/or indirect mechanism such as induction of cytokines, resulting in the anergic state of T cells to further stimulation in vitro. Furthermore, the induction of IL-2Rα expression was depressed in both CD4+ and CD8+ cells, indicating that the unresponsiveness of PBMC induced by FIV infection is not restricted to CD4+ cells.

The expression of MHC-II on unstimulated feline PBMC, especially on large population of T cells, was reported to be associated with antigen recognition and responsiveness to allogenic MHC. The number of circulating MHC-II+ cells was increased in FIV-infected cats, which is consistent with the data of IL-2Rα expression demonstrated in the present study. Recent reports showed that the
expression of MHC-II on CD8+ cells [15] and monocytes [3] were detected in AIDS patients, and these expression of MHC-II were thought to be associated with immune dysfunctions by HIV.

The inoculation with FIV in vitro also reduced the induction of IL-2Ra expression on PBMC after Con A-stimulation. At present, it is not clear whether FIV virion or component itself affect signal transduction following to Con A-stimulation in the absence of direct infection. In HIV infection, surface envelope protein (gp120) suppressed PHA-induced lymphocyte blastogenesis and down-regulates IL-2Ra expression [11, 14, 26]. Some investigators also reported that there was a sequence homology between HIV env protein and IL-2 [18], suggesting the possible mechanism for the immuno-suppressive potential of HIV by interfering IL-2 activity directly or indirectly. Further studies are required for clarifying the direct effects of inactivated FIV virion and components on the functions of feline PBMC. On the other hand, it is well known that HIV suppress the expression of CD4 [19], however, the suppression of CD4 expression was not observed after FIV inoculation in this experiment.

The present study demonstrated that FIV infection induced hyporeactivity of T cells to mitogen-stimulation, and this anergic state of T cells might be associated with the susceptibility to opportunistic infection in FIV-infected cats.

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