

## Ability of CD8<sup>+</sup> T Cell Anti-Feline Immunodeficiency Virus (FIV) Activity and FIV Proviral DNA Load in Mononuclear Cells in FIV-Infected Cats

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(Received 8 June 2004/Accepted 14 September 2004)

**ABSTRACT.** We investigated the relationship between CD8<sup>+</sup> T cell anti-feline immunodeficiency virus (FIV) activity and FIV proviral DNA load integrated in mononuclear cells. The anti-FIV activity and the proviral DNA load were correlated, and the number of proviral DNA copies was high in cats with decreased anti-FIV activity. Particularly, no anti-FIV activity was detected in the cats staged as having an acquired immunodeficiency syndrome (AIDS)-related complex or AIDS, and the number of proviral DNA copies was obviously increased compared to those in the cats in the asymptomatic stage. These results suggest that decreased anti-FIV activity destroys the control of *in vivo* FIV replication, which leads to an increased proviral DNA load with the progression of the clinical stage of disease.

**KEY WORDS:** CD8<sup>+</sup> T cell, FIV, proviral DNA load.

*J. Vet. Med. Sci.* 67(1): 129–131, 2005

Feline immunodeficiency virus (FIV) is a lentivirus that causes chronic and progressive acquired immunodeficiency syndrome (AIDS) in domestic cats [10]. As in human immunodeficiency virus (HIV) infection, plasma viremia is detected in the early stage (acute phase) of FIV infection, followed by a long clinically asymptomatic carrier (AC) stage [9].

As virus-specific cytotoxic T cells (CTLs), CD8<sup>+</sup> T cells damage FIV-infected cells, and suppress FIV replication. FIV-specific CTL response was observed following experimental infection with FIV prior to the onset of humoral immunity [1, 11]. In addition, non-cytolytic CD8<sup>+</sup> T cells, which suppress virus replication in a non-MHC-restricted manner by the secretion of soluble factors, have been observed in the AC stages of FIV infection [2–4, 6, 9]. Cell-mediated immune responses chiefly involving these CD8<sup>+</sup> T cells appear to maintain the AC stage of FIV infection.

When host cells are infected with FIV, viral RNA undergoes reverse transcription to DNA, which is mediated by reverse transcriptase, and is integrated into host cell DNA as a provirus, as with other lentiviruses. In this pattern of infection, FIV persists in the host cell DNA as a provirus.

In this study, to further understand the role of CD8<sup>+</sup> T cell anti-FIV activity in the development of AIDS in FIV infection, we investigated the relationship between the CD8<sup>+</sup> T cell anti-FIV activity and the FIV proviral DNA load integrated in lymphocytes.

Nine experimentally FIV-infected cats (TN2, TN4, TN6, TN10, TN11, TN19, NA8, NA9, and NA10) were used in this study. Table 1 shows infected FIV strains, years post-infection, CD4<sup>+</sup> cell counts and CD4/CD8 cell ratios. TN2 and TN6 exhibited a marked loss of body weight, anemia, stomatitis, and symptoms of recurrent respiratory infection, and were staged as having an AIDS-related complex or AIDS of FIV infection. The other 7 animals were clinically

healthy, and were staged as AC of FIV infection.

Mononuclear cells were collected from blood, spleen, and lymph nodes from these cats, and the number of FIV proviral DNA copies integrated in the mononuclear cells and CD8<sup>+</sup> T cell anti-FIV activity were measured. The number of FIV proviral DNA copies was measured by quantitative competitive-polymerase chain reaction (QC-PCR) using DNA competitors consisting of the region of the FIV gag gene. QC-PCR was performed by the method of Hohdatsu *et al.* [5].

CD8<sup>+</sup> T cell-depleted mononuclear cells and unfractionated mononuclear cells were cultured to detect CD8<sup>+</sup> T cell anti-FIV activity according to the method of Hohdatsu *et al.* [8]. The culture supernatants were monitored for FIV p24 antigen at 3-day intervals. The anti-FIV activity of CD8<sup>+</sup> T cells was determined by comparing the produced FIV p24 antigen from CD8<sup>+</sup> T cell-depleted mononuclear cells and those of unfractionated mononuclear cells by enzyme-linked immunosorbent assay (ELISA) optical density (OD) values at 3 time points: on the day when OD peaked, 3 days

Table 1. Peripheral CD4<sup>+</sup> cell counts and CD4/CD8 cell ratio in FIV-infected cats

Cat	FIV strain	Years post-infection	Peripheral CD4 <sup>+</sup> cell counts/ $\mu$ l <sup>a)</sup>	CD4/CD8 cell ratio
TN4	Aomori-II	2.0	464	0.87
TN10	Aomori-II	4.1	1209	1.00
TN11	Aomori-II	4.1	534	0.88
TN19	Aomori-II	4.1	234	0.72
NA8	Aomori-II	4.1	158	0.89
NA9	Aomori-II	4.1	614	0.88
NA10	Aomori-II	2.5	1593	1.26
TN2	Shizuoka	4.9	112	0.51
TN6	Fukuoka	4.1	80	0.68

a) Lymphocyte subset analysis was performed by the method of Hohdatsu *et al.* [8].

Table 2. CD8<sup>+</sup> T cell anti-FIV activity and FIV proviral DNA load in mononuclear cells in FIV-infected cats

Clinical stage	Cat	Organ	Copies of FIV proviral DNA	Anti-FIV activity
Asymptomatic	TN4	blood	10 <sup>4.0</sup>	±
		spleen	10 <sup>4.5</sup>	±
		lymph node	10 <sup>4.0</sup>	—
	TN10	blood	10 <sup>3.0</sup>	±
		spleen	10 <sup>4.0</sup>	±
		lymph node	10 <sup>3.0</sup>	—
	TN11	blood	10 <sup>5.0</sup>	±
		spleen	10 <sup>5.0</sup>	±
		lymph node	10 <sup>5.0</sup>	—
	TN19	blood	10 <sup>3.5</sup>	+
		spleen	10 <sup>4.0</sup>	+
		lymph node	10 <sup>3.5</sup>	+
	NA8	blood	10 <sup>3.0</sup>	±
		spleen	10 <sup>3.0</sup>	+
		lymph node	10 <sup>3.5</sup>	+
	NA9	blood	10 <sup>4.0</sup>	+
		spleen	10 <sup>4.0</sup>	+
		lymph node	10 <sup>3.0</sup>	ND
	NA10	blood	10 <sup>4.0</sup>	±
		spleen	10 <sup>4.0</sup>	±
		lymph node	10 <sup>3.5</sup>	ND
AIDS-related complex or AIDS	TN2	blood	10 <sup>6.5</sup>	—
		spleen	10 <sup>6.0</sup>	—
		lymph node	10 <sup>6.0</sup>	—
	TN6	blood	10 <sup>6.0</sup>	—
		spleen	10 <sup>6.5</sup>	—
		lymph node	10 <sup>6.0</sup>	—

before and 3 days after the peak. The ability of anti-FIV activity was classified into 3 groups, (+), (±), (—). Specifically, anti-FIV activities (+), (±), and (—) were defined as indicating FIV inhibition rates of more than 90% at all 3 time points, at 1 or 2 time points, and at no time points, respectively.

Table 2 shows the CD8<sup>+</sup> T cell anti-FIV activities and numbers of FIV proviral DNA copies contained in 1 µg of DNA of mononuclear cells collected from blood, spleen, and lymph nodes in each cat. The number of FIV proviral DNA copies integrated in mononuclear cells did not vary among the organs in individual animals. However, the copy number was obviously higher in cats TN2 and TN6, staged as having an AIDS-related complex or AIDS, than in the other 7 animals staged as AC. No CD8<sup>+</sup> T cell anti-FIV activity was detected in mononuclear cells from blood, spleen, or lymph nodes in cats TN2 and TN6. As for the 7 cats staged AC, the cats with strong (+) anti-FIV activity in blood mononuclear cells also exhibited strong activity in mononuclear cells from spleen and lymph nodes. No anti-FIV activity was detected in lymph nodes in TN4, TN10, or TN11. The CD8<sup>+</sup> T cell anti-FIV activity and the number of proviral DNA copies are compared among the organs in Fig. 1. In all mononuclear cells from blood, spleen, and lymph nodes, the copy number of integrated FIV proviral DNA tended to decrease with an increase in the anti-FIV activity.

We previously reported that (1) CD8<sup>+</sup> T cell non-cytolytic anti-FIV activity is more effectively induced when CD8<sup>+</sup> T cells are in direct contact with the target cells and when CD8<sup>+</sup> T cells are induced by autologous CD8<sup>+</sup> T cells rather than by allogeneic CD8<sup>+</sup> T cells, and that the activity is mediated by soluble factor(s) produced by CD8<sup>+</sup> T cells

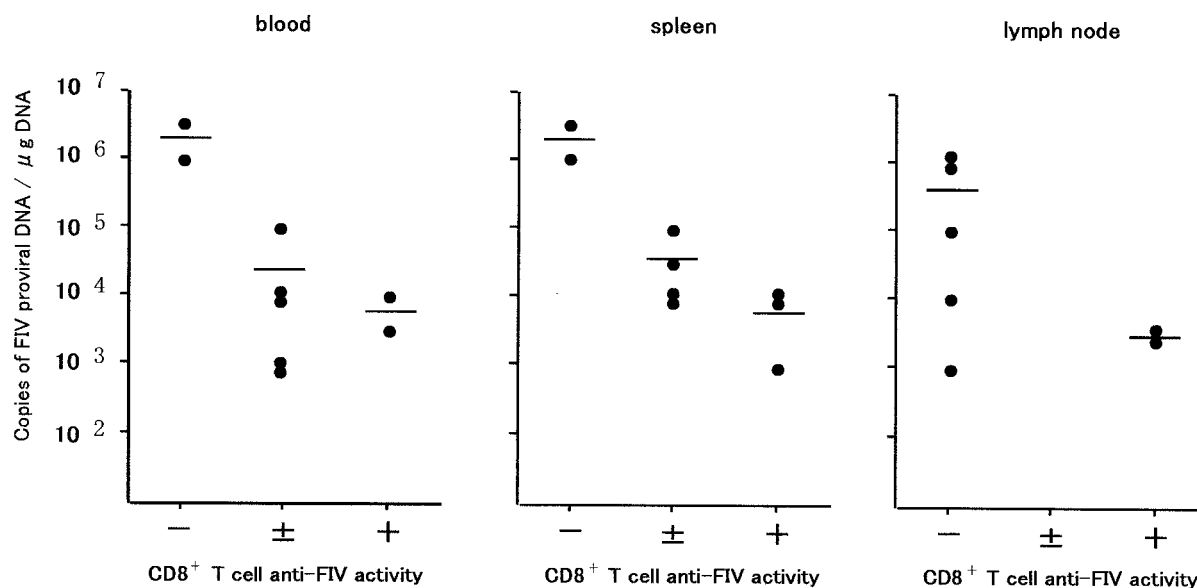


Fig. 1. The relationship between CD8<sup>+</sup> T cell anti-FIV activity and FIV proviral DNA load in mononuclear cells. Bars represent arithmetic mean for number of FIV proviral DNA copies.

[6]; (2) the soluble factor(s) produced by CD8<sup>+</sup> T cells of FIV-infected cats inhibits FIV replication by a non-cytolytic mechanism at the level of FIV mRNA synthesis from the FIV proviral DNA [5]; (3) CD8<sup>+</sup> T cell-mediated inhibition of the synthesis of FIV viral RNA from FIV proviral DNA causes interference with the following exogenous FIV infection (superinfection)[7]; and (4) CD8<sup>+</sup> T cell anti-FIV activity has an important role in plasma viremia and the maintenance of peripheral CD4<sup>+</sup> cell counts [8]. In this study, the CD8<sup>+</sup> T cell anti-FIV activity and the number of FIV proviral DNA copies integrated in mononuclear cells were correlated, and the number of FIV proviral DNA copies was high in cats with decreased anti-FIV activity. Particularly, no CD8<sup>+</sup> T cell anti-FIV activity was detected in the cats staged as having an AIDS-related complex or AIDS, and the number of FIV proviral DNA copies was obviously increased compared to those in the cats in the asymptomatic stage.

The results of this study suggest that decreased CD8<sup>+</sup> T cell anti-FIV activity destroys the control of *in vivo* FIV replication, which leads to an increased number of FIV proviral DNA copies with the progression of the clinical stage of disease. CD8<sup>+</sup> T cell-mediated immune response plays important roles in the maintenance of the asymptomatic stage in FIV-infected cats.

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