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# Decline in CD4$^+$ Cell Numbers in Cats with Naturally Acquired Feline Immunodeficiency Virus Infection

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**T-cell subsets were studied by fluorescence-activated cell sorter analysis in 57 feline immunodeficiency virus (FIV)-seropositive cats with naturally acquired FIV infection to see whether CD4$^+$-CD8$^+$ alterations were comparable to those observed in human immunodeficiency virus-infected patients. CD4$^+$ values were decreased and CD8$^+$ values were increased. The CD4$^+$/CD8$^+$ ratio was reduced to 1.6, compared with 3.3 in 33 FIV-seronegative control cats. Variance analysis of data showed a significant influence of FIV seropositivity, sex, and spaying of female cats on CD4$^+$ values. CD8$^+$ values were significantly influenced by FIV seropositivity, age, and breed. These findings indicate a similarity between FIV and human immunodeficiency virus infections, as far as alterations of T-cell subsets are concerned.**

Feline immunodeficiency virus (FIV), first isolated in California (10), is a typical lentivirus that replicates preferably in feline T-lymphoblastoid cells and is structurally similar to human immunodeficiency virus (HIV). Experimental infection of cats induces transient fever, neutropenia, and lymphadenopathy. Following recovery from the initial phase, cats become lifelong carriers of the virus. One year or more after natural infection, cats may develop a terminal AIDS-like phase (12). Hematologic manifestations of FIV infection, including anemia, lymphopenia, neutropenia, and thrombocytopenia, as well as hyperplasia of individual cell lineages and dysmorphic features in bone marrow, are also similar to those in HIV-seropositive humans (14).

Strong similarities between FIV infection and the human AIDS complex have been shown, not only in virus structure and clinical symptoms but also in epidemiological manifestations (12, 15).

The human counterpart, HIV, is known to infect predominantly CD4$^+$ T-helper lymphocytes and cells of the monocyte-macrophage lineage (3, 8; for a review, see reference 13). Gradual reduction in both the percentage and the absolute number of CD4$^+$ T cells is one of the most striking immunological consequences of HIV infection.

Recently, monoclonal antibodies (MAb) against cat CD4 (1) and CD8 (7) homologs have been developed. We have examined peripheral blood lymphocytes from FIV-positive domestic cats with naturally acquired infection (as judged by seroconversion) by using these anti-CD4 and anti-CD8 MAb. We found a variable reduction of CD4$^+$ lymphocytes and an increased proportion of CD8$^+$ lymphocytes in these cats, as already described in a brief report on 20 FIV-seropositive cats (5).

## MATERIALS AND METHODS

**Animals.** Fifty-seven domestic cats found to be spontaneously FIV antibody seropositive (by Pet Check) following naturally acquired infection (all but two were seronegative for feline leukemia virus [FeLV] by Leukassay FII) were used in the study. Of the 57, 6 were pedigree cats (Table 1). They were between 1 and 14 years old, including 26 neutered males, 5 intact males, 21 spayed females, and 5 intact females (Table 2). Their most prominent clinical signs were chronic infections of the mouth, chronic upper respiratory infections, fever, chronic infections of the skin, inappetence, weight loss, and in some cats, neurological signs or uremia. Control cats were FIV and FeLV seronegative without signs of illness. Of the 33 controls, 17 were pedigree cats (Table 1); they were 1 to 14 years old and included 11 neutered males, 6 intact males, 7 intact females, and 9 spayed females (Table 2).

**Cell preparation.** Peripheral blood mononuclear cells were isolated by centrifugation (15 min, 1,500 × *g*) over a Percoll gradient ($d$ = 1.076). The resulting cell suspension contained mononuclear cells and 5 to 10% granulocytic cells.

**Antibodies.** Mouse MAb Fel7 recognizes the feline CD4 homolog (1), and mouse MAb FT2 identifies a feline CD8 homolog (7). B lymphocytes were stained with a commercially available phycoerythrin-conjugated goat anti-cat immunoglobulin (Southern Biotechnology Associates, Birmingham, Ala.). Mouse MAb were detected with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Jackson Immuno Research Laboratories, West Grove, Pa.).

TABLE 1. Demographic data: breed distribution

| Cats | No. (%) FIV seropositive | No. (%) FIV seronegative | Total no. (%) |
|---|---|---|---|
| Domestic | 51 (89.5) | 16 (48.5) | 67 (74.4) |
| Persian | 2 (3.5) | 5 (15.2) | 7 (7.8) |
| Siamese | 2 (3.5) | 1 (3.0) | 3 (3.3) |
| Oriental shorthair | 0 (0) | 6 (18.2) | 6 (6.7) |
| Abyssinian | 0 (0) | 1 (3.0) | 1 (1.1) |
| Persian × Oriental shorthair | 1 (1.75) | 0 (0) | 1 (1.1) |
| Abyssinian × domestic | 1 (1.75) | 4 (12.1) | 5 (5.6) |
| Pedigree total | 6 (10.5) | 17 (51.5) | 23 (25.6) |
| Total | 57 (100) | 33 (100) | 90 (100) |

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TABLE 2. Demographic data: age distribution by sex and castration

| Cats | No. (mean, range) FIV seropositive | No. (mean, range) FIV seronegative | Total no. (mean, range) |
|---|---|---|---|
| Total | 57 (6.3, 0.4–14) | 33 (5.3, 1–14) | 90 (5.9, 0.4–14) |
| All males | 31 (6.8, 2–14) | 17 (5.5, 2–14) | 48 (6.4, 2–14) |
| All females | 26 (5.6, 0.4–14) | 16 (5.0, 1–12) | 42 (5.2, 0.4–14) |
| Neutered males | 26 (6.8, 2–13) | 11 (6.1, 2–14) | 37 (6.6, 2–14) |
| Unneutered males | 5 (7.4, 2–14) | 6 (4.5, 2–10) | 11 (5.8, 2–14) |
| Spayed females | 21 (6.1, 2–12) | 9 (6.2, 2–12) | 30 (6.2, 2–12) |
| Unspayed females | 5 (3.3, 0.4–13) | 7 (3.4, 1–8) | 12 (3.4, 0.4–13) |

**Immunofluorescence staining.** Two-color immunofluorescence analysis was performed with phycoerythrin-conjugated goat anti-cat immunoglobulin for demonstration of B cells together with either Fel7 or FT2. Cells ($2.5 \times 10^5$/50 μl) were incubated with Fel7, FT2, or an irrelevant mouse anti-rat T-helper MAb (W3/25; Serotec, Wiesbaden, Germany) for negative controls; washed; and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin together with phycoerythrin-conjugated goat anti-cat immunoglobulin. Lymphocytes were analyzed by flow cytometry (FACScan; Becton Dickinson, Mountain View, Calif.). Propidium iodide-positive dead cells were excluded, and nonlymphoid cells were gated out by a combination of forward- and side-light scatter.

**Statistics.** Lymphocyte percentages were stratified by possible confounders and effect modifiers (sex, age, clinical stage, and general condition), and the various subgroups were compared with nonparametric tests (Wilcoxon and Kruskal-Wallis). On the basis of these results, general linear models were fitted for the variables of interest.

## RESULTS

Fluorescence-activated cell sorter analyses of peripheral blood lymphocytes of healthy seronegative cats revealed that 38.7% were $CD4^+$ cells and 13.4% were $CD8^+$ cells (Table 3), resulting in a $CD4^+/CD8^+$ ratio of 3.3, whereas FIV-seropositive cats had 29.8% $CD4^+$ and 22.3% CD8 labelled lymphocytes, all of them negative for feline immunoglobulin. The $CD4^+/CD8^+$ ratio was 1.6. The differences between the values of FIV-seropositive and -seronegative cats are highly significant ($P = <0.001$). The percentages of immunoglobulin-positive B lymphocytes in seropositive cats were not different from those of seronegative animals.

Figures 1 and 2 show the broad variation of $CD4^+$ and $CD8^+$ lymphocytes in both FIV antibody-seropositive and -seronegative cats. Absolute numbers of $CD4^+$ and $CD8^+$ cells gave results similar to the percentages (data not shown), but the increase in $CD8^+$ cells was less prominent than the decrease in $CD4^+$ cells.

Distributions of the sexes are listed in Tables 4 and 5. The numbers of $CD4^+$ lymphocytes in FIV-seronegative male cats were significantly lower ($P = 0.017$) than those in females. Unspayed females had a significantly higher per-

TABLE 3. Percentages of $CD4^+$ and $CD8^+$ cells and $CD4^+/CD8^+$ ratios of FIV-seropositive and -seronegative cats

| FIV status (no. of cats) | Mean % of $CD4^+$ cells ± SD | Mean % of $CD8^+$ cells ± SD | Mean $CD4^+/CD8^+$ ratio ± SD |
|---|---|---|---|
| Seropositive (57) | 29.8 ± 9.3 | 22.3 ± 9.3 | 1.6 ± 1.0 |
| Seronegative (33) | 38.7 ± 9.81 | 13.4 ± 4.3 | 3.3 ± 1.4 |
| $P$ value ($FIV^+$ vs $FIV^-$) | 0.0001 | <0.0001 | <0.0001 |

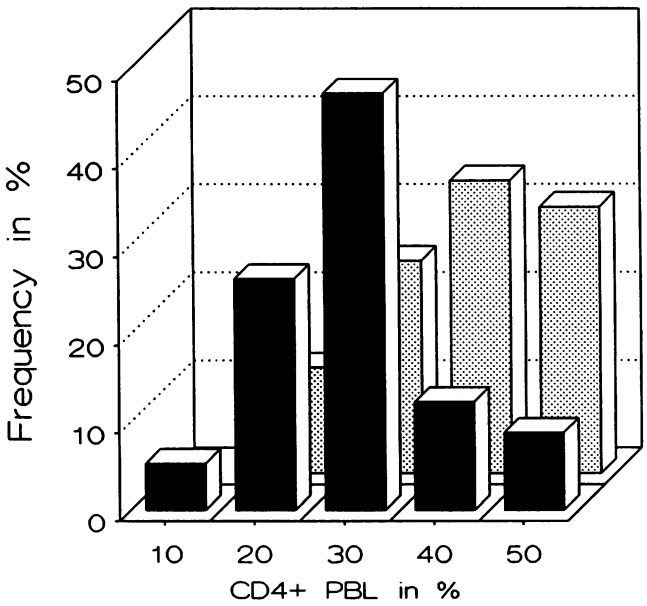


FIG. 1. Frequency of percentages of $CD4^+$ peripheral blood lymphocytes (PBL) in 57 FIV-seropositive (■) and 33 FIV-seronegative (▦) cats.

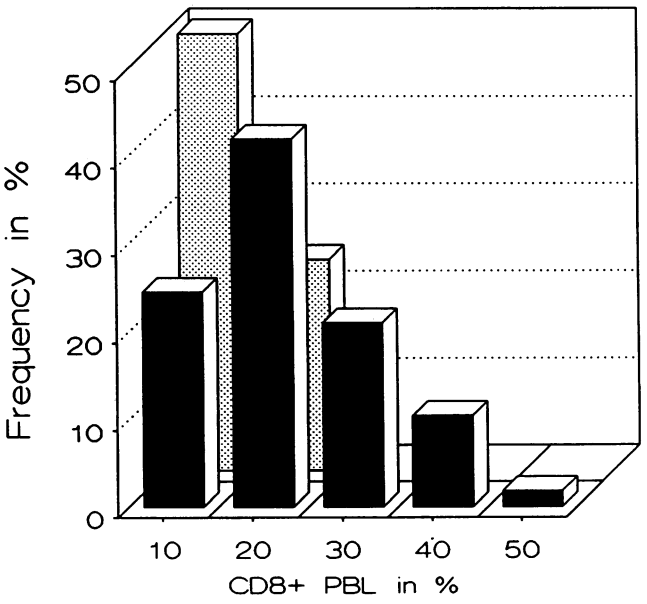


FIG. 2. Frequency of percentages of $CD8^+$ peripheral blood lymphocytes (PBL) in 57 FIV-seropositive (■) and 33 FIV-seronegative (▦) cats.

TABLE 4. Percentages of $CD4^+$ and $CD8^+$ cells and $CD4^+/CD8^+$ ratios of FIV-seronegative cats stratified by sexes

| Group (no. of cats) | Mean % of $CD4^+$ cells ± SD | Mean % of $CD8^+$ cells ± SD | Mean $CD4^+/CD8^+$ ratio ± SD |
|---|---|---|---|
| All males (17) | 34.8 ± 8.8 | 13.7 ± 5.0 | 2.9 ± 1.2 |
| All females (16) | 42.9 ± 8.8 | 13.1 ± 3.2 | 3.6 ± 1.5 |
| *P* value (male vs female) | 0.017 | 0.970 | 0.170 |
| Neutered males (11) | 34.5 ± 9.2 | 13.5 ± 5.2 | 3.0 ± 1.3 |
| Unneutered males (6) | 35.5 ± 8.1 | 14.2 ± 4.6 | 2.7 ± 1.0 |
| *P* value (neutered vs unneutered males) | 0.685 | 0.721 | 0.960 |
| Spayed females (9) | 38.6 ± 8.5 | 14.6 ± 2.4 | 2.7 ± 0.9 |
| Unspayed females (7) | 48.4 ± 5.3 | 11.1 ± 3.2 | 4.8 ± 1.4 |
| *P* value (spayed vs unspayed females) | 0.023 | 0.087 | 0.015 |
| *P* value (unneutered males vs unspayed females) | 0.012 | 0.141 | 0.015 |

centage ($P = 0.023$) of $CD4^+$ cells than did spayed females and also had significantly higher ($P = 0.012$) $CD4^+$ values than uncastrated males (Table 4). The numbers of $CD4^+$ lymphocytes in FIV-seropositive uncastrated females were significantly higher than those in spayed females ($P = 0.003$) and uncastrated males ($P = 0.047$) (Table 5).

Table 6 shows that the levels of $CD4^+$ and $CD8^+$ cells and the $CD4^+/CD8^+$ ratios of FIV-seropositive males and females differed significantly from those of FIV-seronegative male and female cats (all $P < 0.02$).

When FIV-seropositive cats were classified by clinical stages as I (asymptomatic), II (AIDS-related complex), or III (AIDS as defined in reference 14), no significant difference

TABLE 5. Percentages of $CD4^+$ and $CD8^+$ cells and $CD4^+/CD8^+$ ratios of FIV-seropositive cats stratified by sex

| Group (no. of cats) | Mean % of $CD4^+$ cells ± SD | Mean % of $CD8^+$ cells ± SD | Mean $CD4^+/CD8^+$ ratio ± SD |
|---|---|---|---|
| All males (31) | 28.9 ± 8.6 | 22.0 ± 9.3 | 1.66 ± 1.1 |
| All females (26) | 31.0 ± 10.1 | 22.7 ± 9.4 | 1.6 ± 0.9 |
| *P* value (males vs females) | 0.382 | 0.810 | 0.829 |
| Neutered males (26) | 28.0 ± 8.7 | 21.5 ± 9.2 | 1.7 ± 1.2 |
| Unneutered males (5) | 33.4 ± 6.5 | 24.2 ± 9.7 | 1.7 ± 1.0 |
| *P* value (neutered vs unneutered males) | 0.170 | 0.590 | 0.872 |
| Spayed females (21) | 27.9 ± 8.4 | 22.9 ± 9.4 | 1.5 ± 0.8 |
| Unspayed females (5) | 43.8 ± 5.3 | 22.0 ± 9.5 | 2.3 ± 0.8 |
| *P* value (spayed vs unspayed females) | 0.003 | 0.896 | 0.051 |
| *P* value (unneutered males vs unspayed females) | 0.047 | 0.676 | 0.403 |

TABLE 6. *P* values of the differences between FIV-seropositive and -negative male and female cats

| Sex | *P* value[a] for: | | |
|---|---|---|---|
| | $CD4^+$ cells | $CD8^+$ cells | $CD4^+/CD8^+$ ratio |
| Male | 0.023 | 0.002 | 0.006 |
| Female | 0.0009 | 0.0004 | 0.0001 |

[a] All comparisons were of FIV-seropositive versus FIV-seronegative cats.

was observed for $CD4^+$ or $CD8^+$ values or the $CD4^+/CD8^+$ ratio (Table 7).

Therefore, FIV-seropositive cats were classified by general condition. Cats suffering from either stomatitis, rhinitis, or conjunctivitis without further signs of illness, such as fever, inappetence, or changed behavior, were classified as being in "normal general condition." Those cats with at least two symptoms or more severe illness were grouped as being in "disturbed general condition." When FIV-seropositive cats were classified by this scheme, the $CD4^+$ lymphocyte values of cats grouped as being in disturbed general condition were significantly lower ($P = 0.004$) than those of cats in normal general condition (Table 8).

## DISCUSSION

Values of 38.7% ± 9.7% $CD4^+$ lymphocytes in FIV-seronegative control cats are higher than the 25% ± 5% reported elsewhere (1). Our 13.4% ± 4.3% $CD8^+$ lymphocytes is lower than the 15% ± 9% reported by Klotz et al. (7) or the 18% ± 3% reported by Ackley et al. (1). These differences may be caused by different modes of lymphocyte separation and/or gate selection during fluorescence-activated cell sorter analysis.

The high variability of T-lymphocyte subpopulations reflects the well-known high variability of leukocyte and lymphocyte counts. One finding not yet reported is the significant difference between male and female cats, which can be attributed above all to the higher $CD4^+$ values of unspayed female cats.

The group of FIV-seropositive cats contains 57 cats spontaneously FIV infected and includes only 2 cats coinfected with FeLV. The $CD4^+$ and $CD8^+$ lymphocyte values of both of these FeLV-seropositive cats did not deviate from those of the major group and were not even among the extreme values of seropositive animals. As shown, the $CD4^+$ and $CD8^+$ values and $CD4^+/CD8^+$ ratios of FIV-seropositive cats differed significantly from those of FIV-seronegative cats. This was also true for absolute cell counts (data not shown). The same was true of FIV-seropositive male and

TABLE 7. Percentages of $CD4^+$ and $CD8^+$ cells and $CD4^+/CD8^+$ ratios of FIV-seropositive cats stratified by clinical stage[a]

| Clinical stage (no. of cats) | Mean % of $CD4^+$ cells ± SD | Mean % of $CD8^+$ cells ± SD | Mean $CD4^+/CD8^+$ ratio ± SD |
|---|---|---|---|
| I (5) | 28.4 ± 14.6 | 16.4 ± 7.7 | 2.0 ± 1.0 |
| II (23) | 29.3 ± 7.1 | 22.7 ± 8.1 | 1.5 ± 0.8 |
| III (29) | 30.5 ± 9.7 | 23.0 ± 10.2 | 1.7 ± 1.1 |
| *P* value (Kruskal-Wallis test) | 0.943 | 0.079 | 0.589 |

[a] As defined in reference 3.

TABLE 8. Percentages of $CD4^+$ and $CD8^+$ cells and $CD4^+/CD8^+$ ratios of FIV-seropositive cats stratified by general condition

| General condition (no. of cats) | Mean % of $CD4^+$ cells ± SD | Mean % of $CD8^+$ cells ± SD | Mean $CD4^+/CD8^+$ ratio ± SD |
|---|---|---|---|
| Disturbed (25) | 26.4 ± 6.8 | 22.8 ± 10.0 | 1.4 ± 0.8 |
| Normal (32) | 32.5 ± 10.2 | 21.9 ± 8.8 | 1.8 ± 1.1 |
| *P* value (disturbed vs normal) | 0.004 | 0.853 | 0.137 |

female cats versus FIV-seronegative male and female cats. Also, in FIV-seropositive cats, the $CD4^+$ values of unspayed females differed significantly from those of spayed females. The latter could be due to the difference in age distribution. However, FIV-seropositive and -seronegative unspayed female cats had similar age profiles.

Variance analysis was performed to control for the influences of sex, age, and breed because of the very heterogeneous collection of patients and control cats.

Variance analysis (Table 9) showed a significant influence of FIV seropositivity ($P = 0.003$) and sex ($P = 0.002$) on $CD4^+$ values; of FIV seropositivity ($P < 0.001$), age ($P = 0.023$), and breed ($P = 0.006$) on $CD8^+$ values; and of FIV seropositivity ($P < 0.001$), breed ($P = 0.007$), and sex ($P = 0.037$) on the $CD4^+/CD8^+$ ratio. When spaying was included as a further variable, variance analysis showed a significant influence of FIV seropositivity ($P = 0.007$) and spaying ($P = 0.006$) on the $CD4^+$ values of all female cats but of FIV seropositivity ($P = 0.002$), age ($P = 0.040$), and breed ($P = 0.048$) on the number of $CD8^+$ lymphocytes and of FIV seropositivity ($P = 0.001$), breed ($P = 0.035$), and spaying ($P = 0.037$) on the $CD4^+/CD8^+$ ratio.

Selective depletion of $CD4^+$ T-helper lymphocytes (6), increased levels of $CD8^+$ T-suppressor cells (4), and a lowered $CD4^+/CD8^+$ ratio (9) are some of the most prominent features of HIV infection in humans. In human AIDS, a correlation between the degree of T-cell subset alteration and clinical stages is well known. In this study, we did not know the duration of FIV infection, because seropositivity of cats with naturally acquired FIV infection was the only parameter for inclusion and cats experimentally infected were not included. Therefore, we looked for correlations between severity of illness and lowered $CD4^+$ or increased $CD8^+$ lymphocytes.

TABLE 9. *P* values of the general linear model fitted for $CD4^+$ and $CD8^+$ cells and the $CD4^+/CD8^+$ ratio

| Group and variable | *P* value for: | | |
|---|---|---|---|
| | % of $CD4^+$ cells | % of $CD8^+$ cells | $CD4^+/CD8^+$ ratio |
| All cats ($n = 90$) | | | |
| FIV | 0.003 | <0.0001 | <0.0001 |
| Age | 0.185 | 0.023 | 0.350 |
| Breed | 0.321 | 0.006 | 0.007 |
| Sex | 0.002 | 0.728 | 0.037 |
| *P* value of F test | <0.0001 | <0.0001 | <0.0001 |
| Female cats ($n = 42$) | | | |
| FIV | 0.007 | 0.002 | <0.001 |
| Age | 0.619 | 0.040 | 0.253 |
| Breed | 0.390 | 0.048 | 0.035 |
| Spaying | 0.006 | 0.741 | 0.037 |
| *P* value of F test | <0.0001 | 0.0004 | 0.0001 |

Clinical staging of FIV-seropositive cats as described in reference 14 did not reveal statistically significant differences. Since clinical stages have not been well defined, we preferred to use a more subjective classification based on general condition. This classification resulted in a significant difference ($P = 0.004$) in the level of $CD4^+$ lymphocytes but not in the level of $CD8^+$ cells or in the $CD4^+/CD8^+$ ratio.

Pathogen-free cats experimentally infected with FIV had a significant inversion of the $CD4^+/CD8^+$ T-cell ratio, but only after being infected for 18 months or more (2). These experimentally infected cats were specific-pathogen-free cats housed in pathogen-free facilities all of their lives. This could be the reason for the less prominent decrease of $CD4^+$ cells in comparison with the cats in this study, which were kept as pets. Secondary infections, for example, with FeLV, play a role in the severity of disease but not in the actual T-cell alterations (11).

Our findings indicate that FIV infection in cats induces abnormalities of the T-helper–T-suppressor subpopulations similar to those in HIV-infected humans. Such evidence for a strong similarity between HIV and FIV infections promotes FIV infection in cats as an excellent animal model to study vaccination and therapeutic approaches for AIDS.

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