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Recombinant FeLV vaccine: long-term protection and effect on course and outcome of FIV infection

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Abstract

The efficacy and the long-term protection of a recombinant feline leukemia virus (FeLV) vaccine were determined in 30 specified pathogen free cats for over 3 years. At the same time, in order to specify the effects of feline immunodeficiency virus (FIV) on the immune system, one half of the cats ($n=15$) were previously infected with the Swiss isolate FIV Zurich 2. The second half of the animals ($n=15$) served as non-infected controls. Eighteen (nine FIV-negative, nine FIV-positive) vaccinated and 12 (six FIV-negative, six FIV-positive) non-vaccinated cats were intraperitoneally challenged with FeLV A. Seventeen of 18 vaccinated cats were protected against persistent viremia, while ten of 12 non-vaccinated controls became infected. An increase of antibodies against FeLV SU was found in all protected cats after the challenge exposure. No difference in vaccine efficacy was found between FIV-negative and FIV-positive animals.

The whole group of cats was observed for over 3 years. There were no further vaccinations during this period. $CD4^+$ and $CD8^+$ cell subsets, clinical outcome and time of survival of the cats were recorded. FIV-negative and FIV-positive animals were kept in two different rooms. However, FeLV-negative and FeLV viremic cats were housed together in both rooms in order to imitate a natural FeLV exposure situation. Anti-recombinant FeLV SU antibodies were measured by enzyme-linked immunosorbent assay. Although a continuous decline of antibodies was found in FeLV vaccinated cats, they remained protected against constant FeLV challenge for over 3 years. FIV infection had a stronger effect on the depression of the $CD4^+$:$CD8^+$ ratio than FeLV infection. Within the group of FIV-positive cats, the FeLV-vaccinated animals had significantly better survival rates as well as better clinical and laboratory parameters. FIV- and FeLV-coinfected cats showed the lowest $CD4^+$:$CD8^+$ ratio, mainly caused by decreased $CD4^+$ lymphocyte counts. $CD8^+$ lymphocytes with strong fluorescence ($CD8^{high}$) disappeared and cells with weak fluorescence ($CD8^{low}$) appeared instead. Pre-

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vention of coinfection by immunizing FIV-positive cats against FeLV infection improved the clinical outcome and prolonged the cat's life expectancy.

Abbreviations

ELISA, enzyme-linked immunosorbent assay; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; SPF, specified pathogen free.

1. Introduction

In 1991 a new recombinant vaccine against feline leukemia virus (FeLV) was introduced containing FeLV SU expressed in *Escherichia coli* (Kensil et al., 1991; Marciani et al., 1991). First experimental studies demonstrated a very high efficacy of this vaccine against intraperitoneal challenge exposure (Clark et al., 1991; Lehmann et al., 1991; Marciani et al., 1991). In a field trial to investigate its safety, no serious systemic reactions were observed and the vaccine was found to be safe (Clark et al., 1991). The goal of the present study was to determine the long-term protection of a single basic FeLV immunization using the recombinant vaccine in a natural exposure situation. Eighteen vaccinated cats were housed together with ten FeLV-positive carrier cats for over 3 years.

In an earlier study, the functioning of the feline immune system during short-term feline immunodeficiency virus (FIV) infection was investigated. For this purpose cats had been infected with FIV Zurich 2. The FIV-infected animals did not show a suppression of the immune system in the early phase of infection (Lehmann et al., 1991). However, FIV infection will lead to a decline of the $CD4^+$:$CD8^+$ ratio of lymphocytes over a period of several months or years (Barlough et al., 1991; Torten et al., 1991). This progresses to the clinical stage and finally to the full-blown immunodeficiency syndrome (Ishida and Tomoda, 1990; Ishida et al., 1992). In this study, the course and outcome of FIV and FeLV infection were observed, and the $CD4^+$ and $CD8^+$ lymphocyte subsets were measured by flow cytometry during the whole 3 year period.

2. Materials and methods

2.1. Animals

Specified pathogen free (SPF) cats (16 female, 14 male) were obtained from Ciba Geigy, Basel, Switzerland. They were housed in groups of 15 in two identical climatized rooms at the Veterinary Faculty of the University of Zurich. All cats were clinically examined weekly and blood samples were collected regularly. Cats that had to be euthanized underwent detailed necropsy and histopathological examination.

2.2. Experimental FIV infection

Fifteen cats were inoculated intraperitoneally at the age of 17 weeks with 1 ml of supernatant collected from FIV Zurich 2 infected lymphocyte culture. FIV infection was

monitored as described (Lutz et al., 1988) by detection of antibodies (enzyme-linked immunosorbent assay (ELISA), Western blot) and by virus isolation from blood lymphocytes.

2.3. Basic FeLV immunization

Twenty-three weeks later, a total of 18 cats (nine FIV-negative, nine FIV-positive) were vaccinated intramuscularly with a recombinant FeLV vaccine (Leucogen®; Virbac Laboratoires, Carros, Nice, France; in the USA, Genetivac®; Pitman-Moore, Washington-Crossing, NJ) twice within 3 weeks. One dose of this vaccine contained 100 μg SU FeLV subtype A expressed in *Escherichia coli* with 2 mg aluminium hydroxide in 1 ml QS21 adjuvant. Antibodies to the FeLV vaccine were measured as described (Lutz et al., 1988) by ELISA, using 0.2 μg recombinant SU for coating and by Western blot with 1 μg gradient purified FeLV per strip. Virus neutralizing activity was determined by focus-inhibition assay on the cloned cat cell line C81 (Lehmann et al., 1991).

2.4. FeLV challenge exposure

All cats were FeLV challenged by intraperitoneal injection 15 weeks after the second vaccination. Each animal received 5 ml of cell culture supernatant containing 10^6 focus forming units of FeLV subtype A. FeLV infection was monitored by ELISA (Lutz et al., 1983), by virus isolation from blood and from bone marrow samples (Lehmann et al., 1991) and by Western blot using gradient purified FeLV grown in FL-74 cells (Theilen et al., 1969). FeLV proteins were detected in tissue sections from necropsied animals by indirect immunoperoxidase assay (Reinacher and Theilen, 1987).

2.5. Long-term observation

The whole group of cats was kept for a period of over 3 years. FIV-negative and FIV-positive animals were housed in two separate rooms. Within these two rooms, however, the FeLV-negative cats had close contact to FeLV viremic animals to imitate a natural FeLV exposure situation. No revaccinations were carried out.

2.6. Flow cytometry

$CD4^+$ and $CD8^+$ lymphocytes were determined by flow cytometry as described (Holznagel et al., 1995). Briefly, whole blood samples were defibrinated mechanically, lysed with hypotonic formic acid and indirectly labelled with anti CD4 or CD8 antibodies. Fluorescein-conjugated $F(ab)_2$ goat anti mouse IgG was used as secondary antibody. The labelled cells were analysed by an EPICS Profile Analyzer (EPICS Division Coulter Immunology, Hialeah, FL, USA).

2.7. Statistics

The ELISA results and $CD4^+$ and $CD8^+$ cell subset counts were analysed for significant differences by the Mann–Whitney *U*-test. Frequencies were compared, using the Fisher

exact test for small numbers. Differences were considered significant if $P < 0.05$ (Sachs, 1984).

3. Results

3.1. Recombinant FeLV vaccine

FIV-negative and FIV-positive FeLV vaccinated cats developed high antibody levels against FeLV SU. FIV-positive cats showed significantly higher antibodies after primary immunization at Day 21 (Lehmann et al., 1991).

There was an increase of anti FeLV SU antibodies in 17 of 18 vaccinated cats and in six of 12 non-vaccinated cats after intraperitoneal FeLV challenge (see Fig. 2). These 17 FeLV vaccinated cats were protected from persistent viremia, while ten of the 12 non-vaccinated cats became persistently viremic (Fig. 1). Two cats of the vaccinated group and two of the non-vaccinated group showed a transient viremia. Results of virus isolation and virus neutralizing antibody assays are published elsewhere (Lehmann et al., 1991). All cats developed antibodies to FeLV core proteins as judged from Western blot analysis performed before and 3 weeks after challenge infection (data not shown). The 'preventable fraction' (Pollock and Scarlett, 1990) of the recombinant FeLV vaccine in our hands was calculated to be 93%.

During 3 years of observation after FeLV challenge exposure, anti FeLV SU antibodies gradually decreased in the vaccinated cats (Fig. 2). No significant difference was detected between FIV-negative and FIV-positive animals. Three years after basic immunization, specific antibodies in the vaccinated cats had dropped to about 50% of the positive control included in every ELISA. In some of the cats the decrease of antibodies was so marked that no antibodies were detectable in our ELISA system. For comparison, the mean antibody level measured after FeLV basic immunization was approximately 150%. In spite of these low antibody levels, all FeLV p27 negative, vaccinated cats remained FeLV negative throughout the whole observation period (Figs. 1(a) and 1(c)). One FeLV vaccinated cat (Cat 263), which initially was FeLV-positive, turned negative after more than 1 year.

In the FeLV non-vaccinated animals periods of increased antibodies to FeLV SU were detected (data not shown). However, all infected animals stayed FeLV p27 antigen ELISA positive (Figs. 1(b) and 1(d)).

3.2. Clinical outcome and survival of infected cats

No severe clinical signs were seen during the whole observation period in any of the surviving cats. Some animals showed chronic stomatitis and gingivitis and loss of weight. During the experiment a total of five cats had to be euthanized (Table 1). Two were FIV-negative. One (Cat 289) was FeLV vaccinated; the other (Cat 264) was not FeLV vaccinated. Both these cats suffered from lymphosarcoma.

Cat 289 was of special interest. It had been constantly FeLV-negative by ELISA of blood samples and in bone marrow culture (Lehmann et al., 1991). In spite of the fact that it was

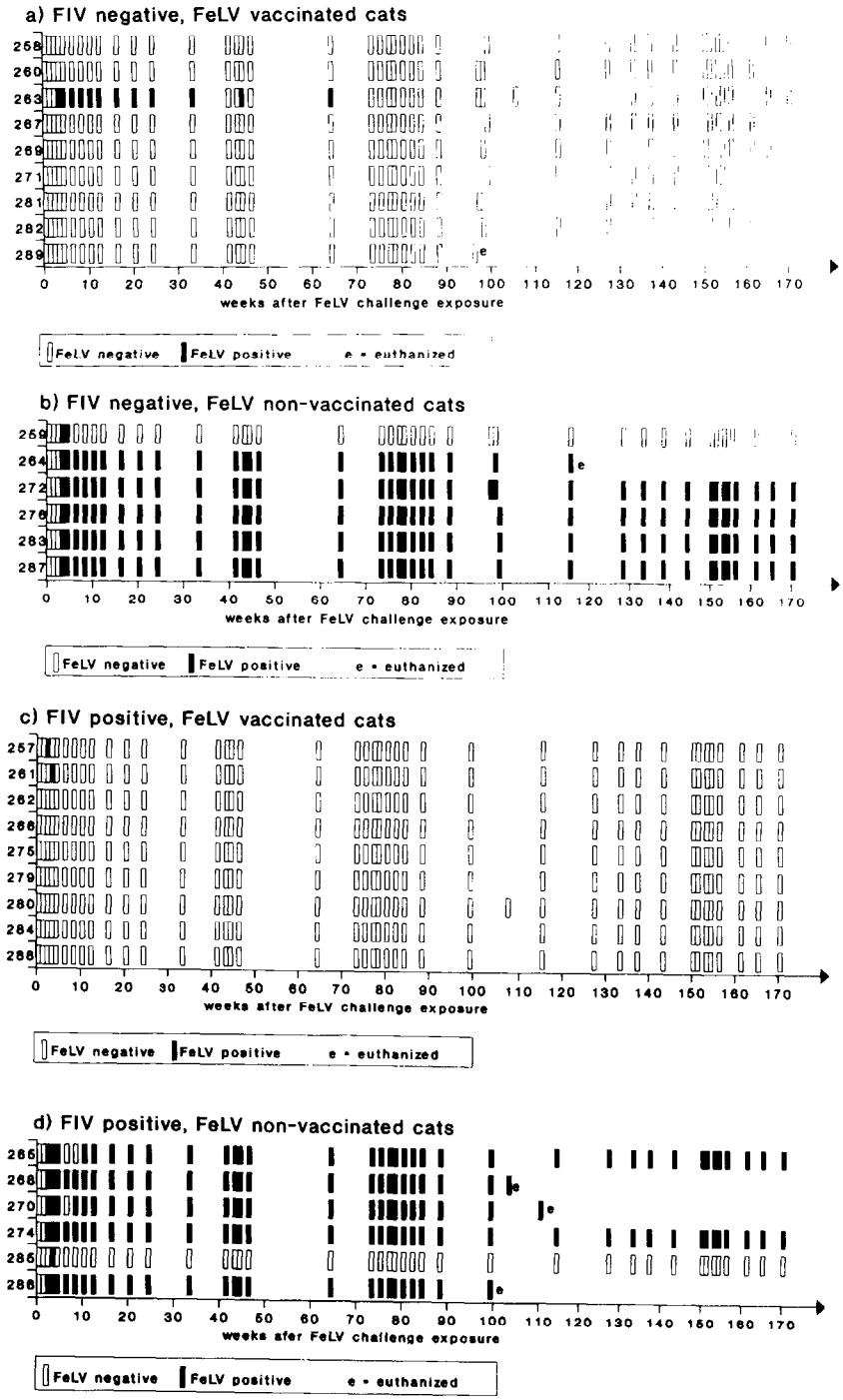

Fig. 1(a–d). Course of FeLV infection. FeLV p27 as determined by ELISA after challenge exposure.

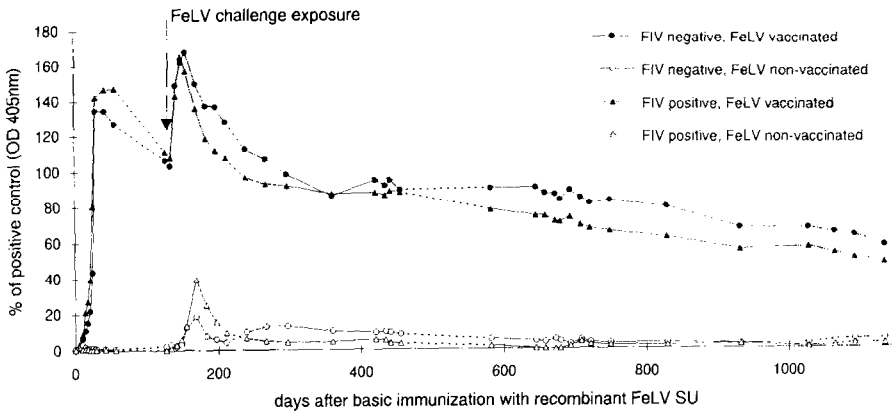

Fig. 2. Development of antibodies to recombinant FeLV SU after one basic FeLV immunization. The ELISA optical density (OD) values are given as the percentage of the value of the positive control serum that was included in every assay.

FeLV-negative at the time of death, FeLV proteins were detected by immunoperoxidase assay in tissue sections of the tumour.

Within the FIV-positive groups, the FeLV vaccinated animals had a significantly better outcome than the FeLV non-vaccinated cats with respect to survival time (Fig. 3) and clinical and laboratory parameters. After 2 years of coinfection, three of five FIV- and FeLV-coinfected cats had to be euthanized because of apathy, severe dehydration and a body temperature of over 40°C. Two of these cats suffered from generalized lymphadenitis with hyperplasia of lymphatic tissues. The third cat additionally showed signs of septicemia (Table 1).

Table 1
Euthanized cats: nature of infection, duration of survival and results of necropsy

Cat No.	Status of blood tests		Duration of survival (years)	Results of necropsy
	FIV	FeLV		
289	–	–	1.7	Generalized lymphosarcoma[a]
268	+	+	2	Generalized necrotising lymphadenitis and splenitis Hyperplasia of all lymphatic tissues
270	+	+	2	Generalized moderate lymphadentitis Hyperplasia of lymphatic tissues
286	+	+	2	Generalized necrotizing lymphadenitis Acute hepatitis with bacterial emboli (septicemia?) and icterus Moderate membranoproliferative glomerulonephritis
264	–	+	2.9	Lymphosarcoma in gut wall, mesenteric lymph nodes, spleen, kidney, omentum and diaphragm

[a]FeLV p27 positive by immunoperoxidase assay.

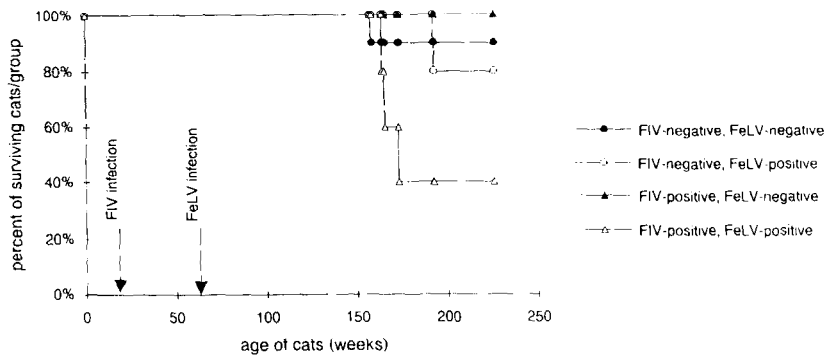

Fig. 3. Percentage of surviving cats with or without FeLV and FIV infection during a 3 year follow up period.

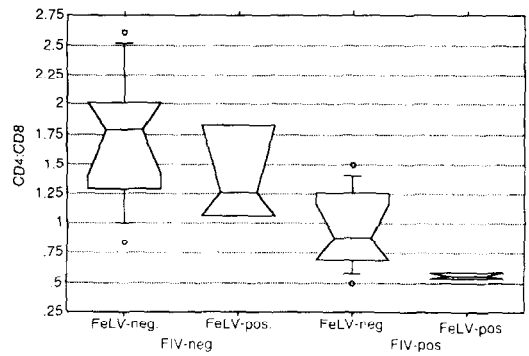

Fig. 4. Box plot of the $CD4^+$:$CD8^+$ ratio of uninfected controls, FeLV-infected cats, FIV-infected cats and FIV- and FeLV-coinfected cats, 4 years after FIV infection and 3.25 years after FeLV infection, respectively.

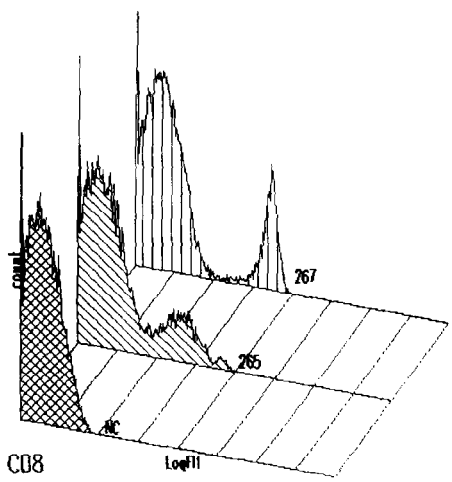

Fig. 5. $CD8^+$ cell subsets determined by flow cytometry of Cat 265 (FIV- and FeLV-coinfected) and Cat 267 (FIV- and FeLV-uninfected) 4 years after FIV infection and 3.25 after FeLV infection, respectively. NC, negative control; log Fl1, fluorescence, logarithnic scale; count, number of cells, linear scale.

3.3. Flow cytometry

Three years after the FeLV challenge exposure, the mean $CD4^+$:$CD8^+$ ratio was higher in the FIV- and FeLV-negative cats, lower in FIV- and FeLV-coinfected cats (Fig. 4). FIV-infected cats had a significantly lower ratio than FIV-negative cats ($P_{MWU} = 0.0012$). The decline of the ratio in FIV-infected cats was mainly caused by low relative and absolute $CD4^+$ lymphocyte counts. FeLV did not cause a decrease in the $CD4^+$:$CD8^+$ ratio within 2.25 years after infection. In FIV- and FeLV-coinfected cats a massive decline of $CD4^+$ lymphocytes was found. Absolute $CD4^+$ numbers were reduced to a minimum of 171 cells μl^{-1}. $CD8^+$ lymphocytes with strong fluorescence ($CD8^{high}$) had almost completely disappeared, while simultaneously cells with distinctly weaker fluorescence ($CD8^{low}$) appeared (Fig. 5).

4. Discussion

4.1. Recombinant FeLV vaccine

A single basic immunization against FeLV infection using a recombinant FeLV SU vaccine protected ten of 12 cats from persistent viremia for a period of over 3 years. An increase of antibodies against FeLV SU after FeLV challenge exposure was found in all vaccinated cats that were protected against FeLV viremia. The booster-like increase of antibodies against SU can be explained by a minimal viremia which, however, was detected in only two of 17 protected cats. Western blot results supported that a very brief viremia existed in all animals. Antibodies against p15, p17 and p27 appeared in all cats after challenge infection, independent of vaccination. Virus replication was immediately controlled by the immune system. This was concluded from the observation that antibodies against SU measured by ELISA reached a maximum only 4 weeks after challenge infection and thereafter steadily decreased. With respect to protection, a very brief period of virus replication may be beneficial against FeLV infection.

Antibodies against FeLV SU gradually decreased during the 3 years following FeLV immunization (Fig. 2) but protection was complete (Fig. 1) in spite of living in a constant FeLV challenge situation. It may be speculated that not only humoral immunity but also cell-mediated immunity must play an important role in the protection found in these cats. The extent of cell-mediated immunity participation or whether both forms are essential for effective protection was not investigated in further detail.

It can be imagined that the protection was prolonged by the FeLV challenge exposure, which may have had an effect similar to a booster immunization. It is not known to what extent challenge and time of exposure is important for protection. However, we feel that the experimental setting mimicked the field situation of vaccination and subsequent challenge exposure.

4.2. Clinical outcome and survival of infected cats

One FeLV vaccinated cat (Cat 263), expected to remain persistently infected with FeLV, turned FeLV-negative about 1 year after FeLV challenge exposure (Fig. 1). Whether reversion to an FeLV-negative status after a viremic phase of such a long duration is frequent

in the field has not, to our knowledge, been investigated in detail. This observation, however, underlines the importance of retesting FeLV-positive cats even many weeks after a positive test because there is a genuine chance that the animal will turn negative after a prolonged viremic phase.

In the FeLV vaccinated group, Cat 289, supposedly protected against FeLV infection, was euthanized because of an FeLV-positve lymphosarcoma. This cat was never positive in ELISA or virus isolation from serum (Fig. 1), nor could any virus be detected in the bone marrow 24 weeks after challenge exposure (Lehmann et al., 1991). There are two possible explanations for the development of this FeLV-positive lymphosarcoma: (1) the virus proteins found in the tumour originated from a brief phase of viremia following the intraperitoneal challenge exposure; during the first few weeks after the challenge exposure the cat's sera were extensively examined but it is still feasible that a very short period of viremia (less than a week) possibly coinciding with a very low virus load could have been missed; (2) the virus originated from a spontaneous infection by close contact with an FeLV-positive cat some time during the 3 year follow up period, and viremia was missed because the intervals between examinations were sometimes longer than a week.

Periods of transient increase of antibodies against recombinant SU were seen in the non-vaccinated, FeLV-infected cats. These increased antibody levels, however, were not sufficient to completely clear the virus from the blood. It was suspected that those periods correlated with a lower virus load in the plasma.

During the 3 years of observation, 10% of the uninfected controls, 20% of the FeLV-monoinfected animals but none of the FIV-monoinfected cats had to be euthanized (Fig. 3). The observation that none of the FIV-monoinfected cats became severely ill or died within the 3 years is of particular interest since it contrasts with results of Ishida et al. (1992), who found 36.4% of FIV-infected animals progressing to the clinical stage within 2 years. In this study, however, it was unknown how long the animals had been infected before they were included in the experiment. Our observation that FIV-monoinfected cats remained healthy for more than 3 years confirm earlier findings in field cats (Lutz et al., 1990), where healthy FIV-infected animals had a mean age of 2.17 years, while FIV-infected cats with disease had a mean age of 6.01 years. It was concluded that the incubation time of FIV infection in field cats in Switzerland must be at least several years.

The survival rate of FeLV-infected cats was surprisingly high (Fig. 3). Normally, the majority of persistently infected animals die within 3 years after onset of viremia (Hardy, 1980). The cats in this study were kept at a high hygienic and ethological standard, which apparently serves to prolong the life of infected cats.

In the FeLV- and FIV-coinfected group, survival rate was lowered markedly (Fig. 3). Three of five cats were euthanized within 2 years (60%). Since it is not yet possible to protect cats reliably from FIV infection other than by complete isolation, it is highly recommended to prevent dual FeLV/FIV infection by vaccinating against FeLV. Healthy FIV-infected cats may be vaccinated effectively with the recombinant FeLV vaccine (Lehmann et al., 1991), which greatly improves the clinical outcome and increases their life expectancy.

4.3. Flow cytometry

The depression of the $CD4^+$:$CD8^+$ ratio in FIV-infected cats was mainly due to decreased $CD4^+$ lymphocyte numbers (relative and absolute). Total $CD8^+$ lymphocyte counts

remained more or less constant. Similar results were observed in long-term FIV-infected cats by Barlough et al. (1991). During FeLV infection, both $CD4^+$ and $CD8^+$ lymphocyte counts decreased slightly more than in the FeLV non-infected animals, but the $CD4^+$:$CD8^+$ ratio remained almost unchanged for 2.25years.

The lowest ratio was seen in FIV- and FeLV-coinfected cats, due to an almost complete loss of their $CD4^+$ lymphocytes. The $CD8^+$ lymphocytes with strong fluorescence ($CD8^{high}$) also disappeared (Fig. 5). Instead, $CD8^+$ cells with distinctly weaker fluorescence ($CD8^{low}$) appeared, as described earlier (Lehmann et al., 1992). There are three possible mechanisms that could explain the rise of these $CD8^{low}$ cells: (1) the CD8 surface marker on the $CD8^+$ lymphocytes is down-regulated; (2) the CD8 marker is masked in a way that binding of antibodies is diminished; (3) during FIV and FeLV infection, cells are released into the blood stream that express low amounts of CD8 markers. In the mouse, large granular lymphocytes with natural killer cell activity have been reported to express low amounts of CD8 markers (Prince et al., 1993). Since no further characterization of $CD8^{low}$ cells was conducted, the cells were not identified. It was interesting to note that the appearance of $CD8^{low}$ cells was accompanied by the decline of $CD8^{high}$ lymphocytes.

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