

# Parameters of Disease Progression in Long-Term Experimental Feline Retrovirus (Feline Immunodeficiency Virus and Feline Leukemia Virus) Infections: Hematology, Clinical Chemistry, and Lymphocyte Subsets

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After several years of latency, feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) cause fatal disease in the cat. The aim of this study was to determine laboratory parameters characteristic of disease progression which would allow a better description of the asymptomatic phase and a better understanding of the pathogenesis of the two infections. Therefore, experimentally infected cats (FIV and/or FeLV positive) and control animals were observed over a period of 6.5 years under identical conditions. Blood samples were analyzed for the following: complete hematology, clinical chemistry, serum protein electrophoresis, and determination of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte subsets. The following hematological and clinical chemistry parameters were markedly changed in the FIV-infected animals from month 9 onwards: glucose, serum protein, gamma globulins, sodium, urea, phosphorus, lipase, cholesterol, and triglyceride. In FeLV infection, the markedly changed parameters were mean corpuscular volume, mean corpuscular hemoglobin, aspartate aminotransferase, and urea. In contrast to reports of field studies, neither FIV-positive nor FeLV-positive animals developed persistent leukopenia, lymphopenia, or neutropenia. A significant decrease was found in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in FIV-positive and FIV-FeLV-positive animals mainly due to loss of CD4<sup>+</sup> lymphocytes. In FeLV-positive cats, both CD4<sup>+</sup> and, to a lesser degree, CD8<sup>+</sup> lymphocytes were decreased in long-term infection. The changes in FIV infection may reflect subclinical kidney dysfunction, changes in energy and lipid metabolism, and transient activation of the humoral immune response as described for human immunodeficiency virus (HIV) infections. The changes in FeLV infection may also reflect subclinical kidney dysfunction and, in addition, changes in erythrocyte and immune function of the animals. No severe clinical signs were observed in the FIV-positive cats, while FeLV had a severe influence on the life expectancy of persistently positive cats. In conclusion, several parameters of clinical chemistry and hematology were changed in FIV and FeLV infection. Monitoring of these parameters may prove useful for the evaluation of candidate FIV vaccines and antiretroviral drugs in cats. The many parallels between laboratory parameters in FIV and HIV infection further support the importance of FIV as a model for HIV.

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are retroviruses of cats which are found worldwide under natural conditions (23, 32, 34, 48, 69). FIV infection leads to an immune suppression and to an AIDS-like syndrome (20, 48). FeLV infection induces anemia, an AIDS-like syndrome, and, less frequently, lymphomyeloproliferative or neurologic disorders (14). Although the fatal outcome of these two retrovirus infections is well known and feared, much less is known about the preceding asymptomatic phase which usually lasts several years. Parameters of clinical chemistry and hematology have been studied in naturally infected cats (19, 57–59, 62, 68). However, the changes documented under field conditions may not necessarily be attributed to the underlying retrovirus infections. A broad variety of factors such as age and living conditions of the animal or other infections may contribute to the observed changes, and the duration of the infection usually is unknown. No information on the course of these parameters in long-term experimental FeLV and FIV infections is available. Therefore, it was the goal of the present

study to monitor the course of as many parameters as possible that can easily be determined in a diagnostic laboratory routine to define those that are associated with FIV and/or FeLV infection. We hoped to determine parameters characteristic of disease progression which would allow a better description of the asymptomatic phase and a better understanding of the pathogenesis of the two infections. In addition, knowledge of parameters of disease progression may be important for evaluation of antiretroviral drugs or vaccines. While efficacious FeLV vaccines are readily available (28, 39), no FIV vaccines have been introduced to the field. Today it appears likely that FIV vaccines that protect against the development of disease but not against infection can be designed (18, 35). Especially in this latter context, monitoring parameters of disease progression will be important.

## MATERIALS AND METHODS

**Study design and cats.** Specific-pathogen-free cats were experimentally infected, observed, and sampled over several years: 15 cats were experimentally infected with FIV strain Zurich 2 (46) at the age of 17 weeks (month 0) by intraperitoneal application of 1 ml of cell culture supernatant containing FIV Zurich 2, and 15 cats served as age-matched FIV-negative controls. In each group, nine cats were vaccinated against FeLV infection with a recombinant FeLV vaccine (28); all cats were subsequently challenged with FeLV by intraperitoneal application of 5 ml of cell culture supernatant containing 10<sup>6</sup> focus-forming units of FeLV subtype A at month 10 (28). FIV was monitored by

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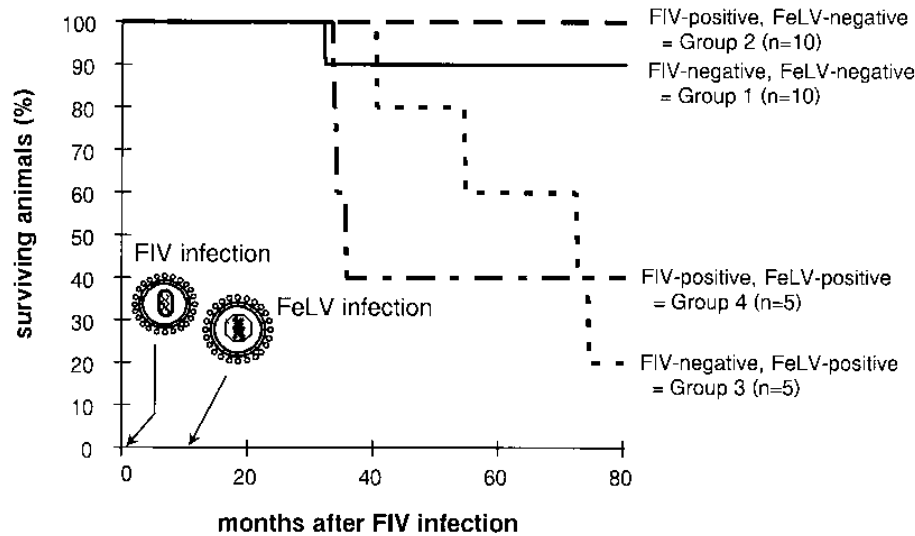


FIG. 1. Survival of cats (percent) after FIV and/or FeLV infection and of controls.

detection of antibodies (by enzyme-linked immunosorbent assay [ELISA] and Western blot [immunoblot]) and by virus isolation from blood lymphocytes (33). FeLV infection was monitored by detection of antibodies by the use of recombinant FeLV surface protein in an ELISA as described previously (28), by detection of antigen p27 by a sandwich ELISA (37), and by virus isolation from blood in a modified clone 81 assay (39). Latent FeLV infection was determined by cultivation of bone marrow samples collected 24 weeks after FeLV infection by procedures described previously (38). For the present study, the cats were subsequently rearranged into four groups by their FeLV and FIV status: group 1, 10 FIV- and FeLV-negative controls; group 2, 10 FIV-positive cats; group 3, 5 persistently FeLV-positive cats; group 4, 5 FIV-positive and persistently FeLV-positive animals. These animals were housed in a special facility of the Department of Internal Veterinary Medicine, University of Zurich. They were fed canned and dry cat food (Whiskas and Brekkies; Effems AG, Zug, Switzerland). All cats were clinically examined weekly, and blood samples were collected regularly. From a total of 74 blood collections over a period of 80 months, the values of nine samplings in which all parameters were measured were statistically evaluated. All time points are given with respect to the start of the experiment, when FIV infection took place. Cats that had to be euthanized for humane reasons underwent detailed necropsy and histopathological examinations.

**Hematology, lymphocyte subsets, clinical chemistry, electrophoresis, and statistics.** Hematology parameters were evaluated in EDTA-blood by routine procedures with a Contraves Autolyzer (AVL AG, Schaffhausen, Switzerland). Differential counts were performed twice manually on 100 leukocytes (WBC). Plasma protein and fibrinogen were determined by a refractometric method. Feline CD4<sup>+</sup>, CD8<sup>+</sup>, and CD5<sup>+</sup> lymphocytes were determined in blood samples by flow cytometry as described before (16). Briefly, whole blood samples were defibrinated and indirectly stained with properly diluted culture supernatants from hybridomas secreting monoclonal antibodies to feline CD4 or CD8 (1, 24). Unconjugated primary antibodies were detected by fluorescein-conjugated F(ab)<sub>2</sub> goat anti-mouse immunoglobulin G (Milan Analytica AG, La Roche, Switzerland). Stained blood samples were lysed with hypotonic formic acid, and the labeled cells were analyzed in a Profile Analyzer (EPICS Division, Coulter Immunology, Hialeah, Fla.). For dual color analysis, feline CD4<sup>+</sup>, CD5<sup>+</sup>, and CD8<sup>+</sup> lymphocytes were stained with directly conjugated (fluorescein isothiocyanate or R-phycoerythrin) murine monoclonal antibodies (Southern Biotechnology Associates Inc., Birmingham, Ala.; 0.25 to 0.5 μg of immunoglobulin G per 10<sup>6</sup> cells). The following biochemical parameters were assayed by automated analysis (Cobas Mira; Hoffmann-La Roche AG, Basel, Switzerland) by standard procedures recommended by the International Federation of Clinical Chemistry as compiled by Tietze (65): bilirubin, glucose, urea, creatinin, protein, albumin, cholesterol, triglyceride, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase, lipase, amylase, sodium, potassium, calcium, and phosphorus. All samples from one collection date were analyzed within one run. Serum protein electrophoresis was based on cellulose-acetate sheets (Boskamp; Nanolab AG, Schlieren, Switzerland). Data obtained from the different groups were displayed or calculated by the box plot method (42). In all figures displaying box plots, the box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median, and the whiskers extend down to the smallest value and up to the largest value. Hematological and biochemical parameters of the three FIV- and/or FeLV-positive groups and the negative control group were analyzed for significant differences ( $P < 0.05$ ) with the SAS (Cary, N.C.) System for Windows 6.10 by the Kruskal-Wallis test (nonparametric

analysis of variance, SAS, proc npar1way wilcoxon). In addition, groups were compared individually with the controls by the Wilcoxon rank sum test (SAS proc npar1way wilcoxon).

## RESULTS

**Clinical outcome and survival.** No severe clinical signs were seen during the whole observation period in any of the surviving cats. Some animals showed mild stomatitis and gingivitis, transient mild diarrhea, and loss of weight. All of the FIV-positive cats survived; four of five cats viremic for FeLV had to be euthanized for humane reasons (Fig. 1). One cat of group 3 had to be euthanized after only 2.5 years (Table 1). At necropsy, all of the four euthanized FeLV-positive cats showed either lymphocytic or myeloid leukemia (Table 1). In summary, 1 of the 10 controls (group 1), four of the five persistently FeLV-positive cats (group 3), and three of the five FIV-FeLV-positive cats (group 4) had to be euthanized (Fig. 1). Necropsy revealed lymphosarcomas in six of the eight cats (Table 1). One cat showed signs of septicemia mediated by immune suppression, and one cat suffered from myeloid leukemia.

**Hematology.** When the hematological parameters of groups 1 to 4 were analyzed by the Kruskal-Wallis test, only a few significant differences were found. They were detected mainly in the erythron: in erythrocyte (RBC) counts ( $P = 0.0379$ ), in packed cell volume (PCV;  $P = 0.0169$ ), and in hemoglobin ( $P = 0.0466$ ) at month 30, in mean corpuscular hemoglobin concentration at month 19 ( $P = 0.0058$ ), in MCV (mean corpuscular volume) at month 64 ( $P = 0.0214$ ), and at different time points in mean corpuscular hemoglobin (MCH; month 30,  $P = 0.0109$ ; month 43,  $P = 0.0501$ ; month 56,  $P = 0.0499$ ). When the three positive groups, groups 2, 3, and 4, were compared individually with group 1, FIV-positive cats had lower PCVs ( $P = 0.0283$ ), lower RBC counts ( $P = 0.0101$ ), and higher MCH values at month 30 ( $P = 0.0254$ ) and higher mean corpuscular hemoglobin concentration values at month 19 ( $P = 0.0011$ ) than the controls. Furthermore, FeLV-positive cats twice had significantly higher MCH values (month 30,  $P = 0.0040$ ; month 43,  $P = 0.0365$ ) (Fig. 2) than the controls, and mean and median MCV scores of group 3 were constantly higher compared with those of group 1 starting 30 months after FIV infection (significantly higher at month 64;  $P = 0.0160$ ) (Fig. 2).

TABLE 1. Euthanized cats: results of necropsy

Cat no.	Status of blood test		FIV (mo) <sup>a</sup>	FeLV (mo) <sup>b</sup>	Results of necropsy
	FIV	FeLV			
289	–	–	32	23	Generalized lymphosarcoma (tissue sections of the tumor were FeLV p27 positive by indirect immunoperoxidase assay)
286	+	+	34	24	Generalized necrotizing lymphadenitis; acute hepatitis with bacterial emboli (septicemia?) and icterus; moderate membranoproliferative glomerulonephritis
268	+	+	34	24	Generalized necrotizing lymphadenitis and splenitis; hyperplasia of all lymphatic tissues
270	+	+	36	26	Generalized moderate lymphadenitis; hyperplasia of lymphatic tissues
264	–	+	40	31	Lymphosarcoma in gut wall, mesenteric lymph nodes, spleen, kidney, omentum, and diaphragm
287	–	+	55	46	Lymphosarcoma in thymus, liver, several lymph nodes, and bone marrow; hyperplasia of all lymphatic tissues
276	–	+	73	63	Myeloid leukemia; hyperplasia of lymphatic tissues
272	–	+	75	66	Lymphosarcoma in thymus, diaphragm, mesenteric lymph nodes, and kidney

<sup>a</sup> Months after FIV infection took place in the experiment.

<sup>b</sup> Months after FeLV infection took place in the experiment.

With the exception of FIV-FeLV-infected cats at month 19 (Fig. 3), no significant differences were found in total WBC counts, although there were trends for lower WBC counts in FIV-, FeLV-, and FIV-FeLV-positive cats at and after month 30 after FIV infection (Fig. 3). Furthermore, no significant differences were found in the absolute differential counts.

**Lymphocyte subsets.** Relative and absolute CD4<sup>+</sup> counts were determined at month 19 and in all samples collected thereafter. They were significantly different between the four groups at month 19 and after month 43 ( $P < 0.05$ ). Comparison of positive groups 2, 3, and 4 individually with the control group 1 revealed lower relative CD4<sup>+</sup> counts in the FIV-positive group 2 compared with that of group 1 at month 19 and at and after month 56 ( $P < 0.05$ ) and lower absolute CD4<sup>+</sup> counts in group 2 starting 56 months after FIV infection ( $P < 0.05$ ) (Fig. 4). Surprisingly, the differences at month 43 were due to lower relative ( $P = 0.0338$ ) and absolute CD4<sup>+</sup> counts ( $P = 0.0085$ ) (Fig. 4) in the FeLV-positive group 3. The lowest relative and absolute CD4<sup>+</sup> counts were found in group 4 (Fig. 4). No significant differences were found in CD8<sup>+</sup> counts. CD4<sup>+</sup>/CD8<sup>+</sup> ratios were significantly different between the four groups of cats at and after month 43 ( $P < 0.05$ ) due to significant lower ratios in group 2 and group 4 ( $P < 0.05$ ) (Fig. 5). The lowest ratios were seen in the FIV-FeLV-positive cats of group 4 (Fig. 5). The decline of the ratio in group 2 was predominantly caused by low CD4<sup>+</sup> lymphocyte counts. It was paralleled by an increase of CD8<sup>+</sup> cells with low fluorescence intensity described earlier (29). Sorted CD8<sup>+</sup> cells with low fluorescence showed a blast-like morphology. Dual color analysis revealed that these cells were CD5<sup>+</sup>. The lowest number of CD4<sup>+</sup> cells (46 cells/ $\mu$ l) was measured at month 61 in FIV-positive cat no. 261 (group 2), which was perfectly healthy at that time.

**Clinical chemistry and serum protein electrophoresis.** Biochemical parameters are to some degree influenced by feeding and the time of blood collection. To minimize such factors, the animals were always fasted for 16 h before blood sampling in the early morning. As the cats became overweight, dry food was restricted after month 30. However, cats of the four different groups always received the same amount and quality of food. Significant differences in biochemical parameters between the FIV-positive cats and the FIV-negative controls are summarized in Table 2. The major trends were the following:

glucose, phosphorus, protein, sodium, triglyceride, and urea were higher and cholesterol was lower in group 2 than in the controls. In the case of protein (Fig. 6), glucose (Fig. 7), and urea, these trends appeared already between 9 and 19 months after FIV infection. Based on electrophoresis of serum proteins, absolute and relative gamma globulin fractions were higher in group 2 (month 19 to 56;  $P < 0.05$ ) (Fig. 6).

The FeLV-positive cats showed persistently higher AST and urea levels (Table 3). In addition, urea was elevated in FIV-FeLV-positive animals (significantly higher at month 19,  $P = 0.0083$ ; at month 30,  $P = 0.0319$ ; and at month 43,  $P = 0.0446$ ).

## DISCUSSION

The aim of this study was to monitor experimental FIV- and/or FeLV-infected cats over a period of many years under well-controlled conditions to better characterize the asymptomatic phases of the infections, obtain more insight into their pathogenesis, and define easily measurable indicators for development of disease. This latter aspect appears to be especially important in conjunction with the efficacy assessment of FIV vaccines and antiretroviral drugs.

**Clinical outcome and survival.** The asymptomatic period of FIV infection in the present study (group 2) without additional cofactors was more than 6 years (Fig. 1), while under field conditions, the asymptomatic phase was estimated to be shorter (20, 36, 72). There are several facts that may explain this apparently long asymptomatic period. (i) The cats in this study were kept under optimal hygienic and ethological conditions. We assume that this might be the most important reason for the prolonged life expectancy compared with that in field cats. (ii) The strain of FIV used, FIV Zurich 2, had been isolated from a 7-year-old male cat suffering from emaciation and therefore may be expected to induce disease. However, the virus was kept in culture for several weeks prior to intraperitoneal inoculation into the cats of groups 2 and 4. The cultivation might have reduced the virulence of this strain. We expect that some animals will show clinical signs of immunodeficiency shortly since CD4<sup>+</sup> counts in some of these cats have dropped below 100 cells/ $\mu$ l.

In contrast to FIV, persistent FeLV infection had a much more severe influence on the life expectancy of infected cats although all cats were kept under the same conditions (Fig. 1).

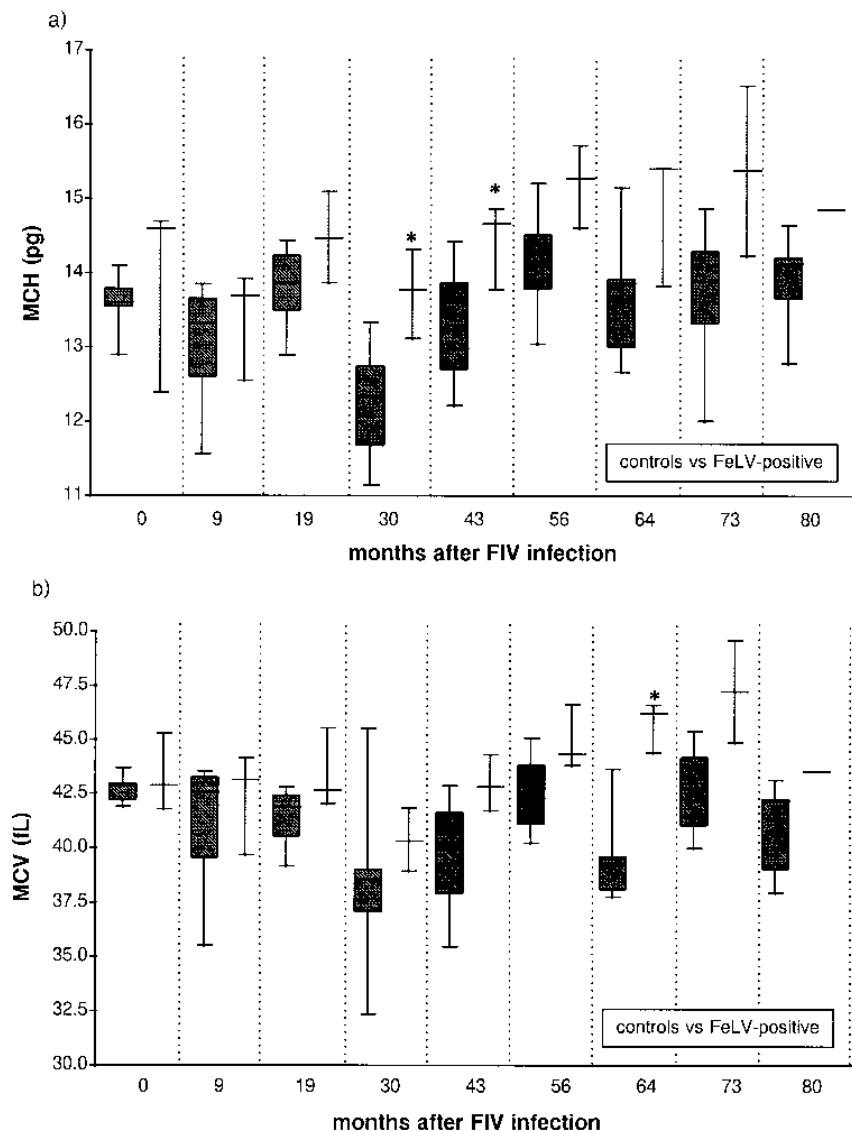


FIG. 2. Box plots of MCH (a) and MCV (b) in controls ( $n = 10$ ; shaded boxes) and FeLV-positive cats ( $n = 5$ ; no boxes shown because of small numbers). \*,  $P < 0.05$  (Wilcoxon rank sum test).

Moreover, it comes as a surprise that four of four cats in this group had neoplastic disease (Table 1). In the field, immunodeficiency and anemia are the predominant forms of chronic FeLV infection. It may be speculated that because of the ideal living conditions, our cats did not succumb to sequelae of immunodeficiency and lived long enough for neoplasia to develop (31 to 66 months of persistent FeLV viremia). In the field, it was estimated that within 3.5 years of persistent FeLV infection, 83% of infected cats would be dead (41).

Pedersen and coworkers (50) have shown that infection with both FIV and FeLV leads to a more severe disease progression than FIV infection alone. Our observation that by month 36, three of five cats with FIV and FeLV infection (group 4) had to be euthanized appeared to confirm this information (Table 1 and Fig. 1). However, in our study, the disease progression was much less pronounced. This may be due to a lower virulence of the FIV and FeLV strains we used. In addition, our cats were first infected by FIV and then by FeLV, which differs from the experimental design of Pedersen and coworkers.

However, it is unknown whether the order of infections is important with respect to the outcome of the clinical course.

One cat (no. 289) is of special interest. This cat obviously had recovered from FeLV infection—it remained FeLV p27 negative for the whole period. After it was euthanized because of tumor development, the tumor was found to be histologically positive for FeLV p27 protein. We speculate that this tumor development might be related to a systemic FeLV infection which went unnoticed. This cat was assigned to the FeLV-negative control group because it was consistently negative for p27 in serum, negative for virus isolation from the serum and bone marrow cultures, developed high virus-neutralizing antibodies, and therefore was considered immune to FeLV infection.

**Hematology.** In earlier studies, FIV infection under field conditions was associated with several hematological abnormalities such as leukopenia, lymphopenia, and neutropenia (17, 62, 72). There are several possible explanations for the absence of significant changes in WBC and differential blood

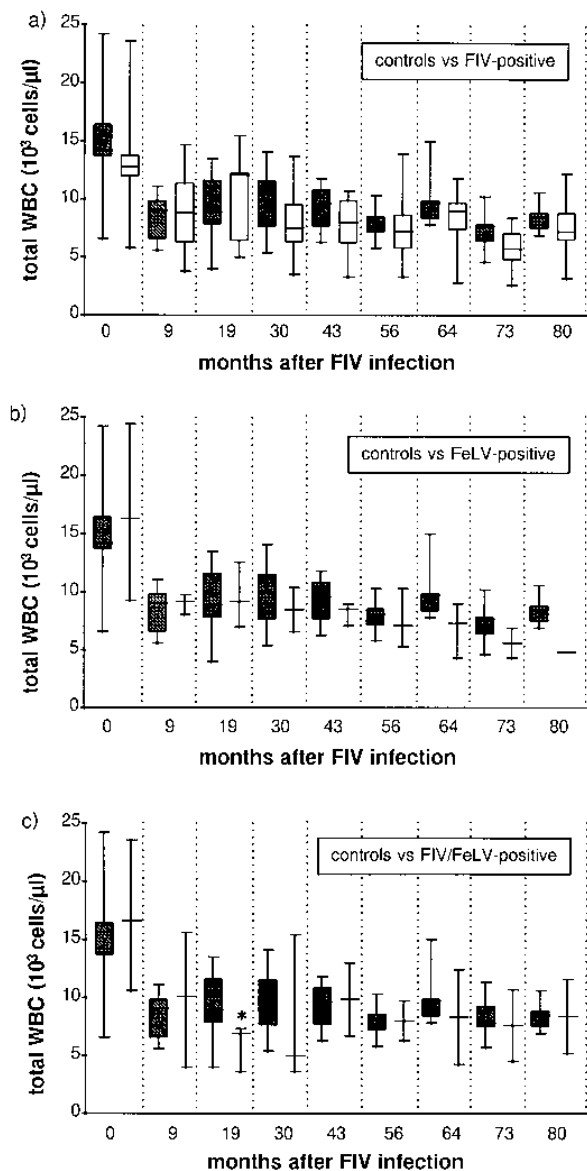


FIG. 3. Box plots of total WBC counts in controls ( $n = 10$ ; shaded boxes) and FIV-positive cats ( $n = 10$ ; open boxes) (a), FeLV-positive cats ( $n = 5$ ; no boxes) (b), and FIV-FeLV-positive cats ( $n = 5$ ; no boxes shown because of small numbers) (c). \*,  $P < 0.05$  (Wilcoxon rank sum test).

cell counts in the present study (Fig. 3). (i) For animal welfare reasons, only a limited number of cats was used in the present study. The chance to observe statistical differences was thus reduced. (ii) The FIV Zurich 2 strain might be of lower virulence than other field strains as mentioned above. (iii) The results in the field studies were obtained from naturally infected ill animals mostly at high risk for FIV infection (17, 62, 72). In the present study, the cats were from the same litters and kept in identical rooms under identical conditions. Therefore, many variables (other pathogens, age, sex, and surroundings), which may play an important role under field conditions, were excluded. It might be speculated that FIV alone will require a longer time to induce changes in WBC counts compared with the field situation where FIV may act synergistically with cofactors. The dramatic drop in WBC counts between

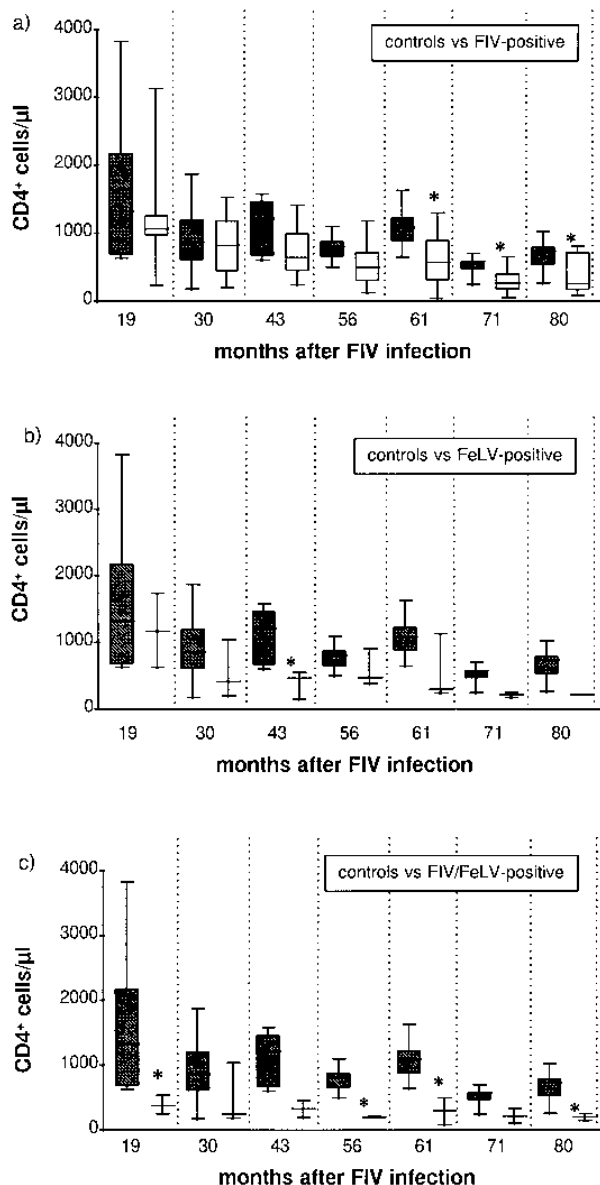


FIG. 4. Box plots of absolute CD4<sup>+</sup> counts in controls ( $n = 10$ ; shaded boxes) and FIV-positive cats ( $n = 10$ ; open boxes) (a), FeLV-positive cats ( $n = 5$ ; no boxes shown because of small numbers) (b), and FIV-FeLV-positive cats ( $n = 5$ ; no boxes shown because of small numbers) (c). \*,  $P < 0.05$  (Wilcoxon rank sum test).

months 4 and 9 (Fig. 3) is due to the natural age-related reduction of WBC counts (49).

Minor changes were found in the erythron. FIV-positive animals showed slightly decreased PCV and RBC values 30 months after infection. This observation was accompanied by a transient increase of MCH. During the early phase of this experiment, blood was collected in weekly intervals. The decreased RBC count and PCV may reflect a somewhat lower capability to regenerate the RBC in FIV-infected cats than in noninfected animals. This, in turn, may be explained by decreases in the frequencies of marrow erythroid progenitors previously reported in some FIV-positive cats (31, 58). FeLV infection led to elevated values of MCH and MCV (Fig. 2), a fact which has been described previously and which may be

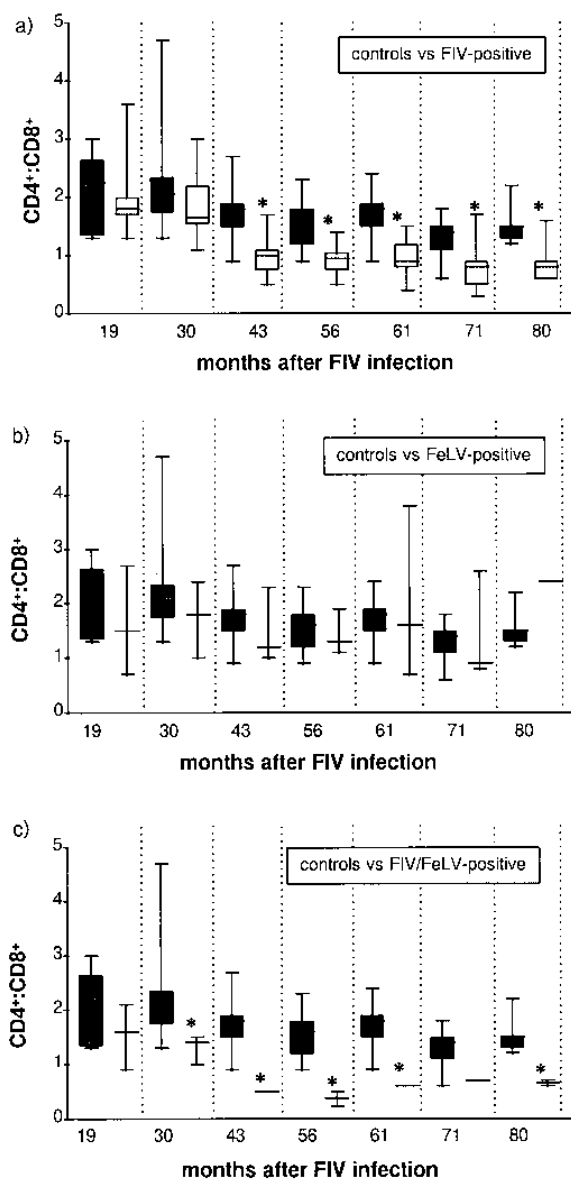


FIG. 5. Box plots of  $CD4^+/CD8^+$  ratio in controls ( $n = 10$ ; shaded boxes) and FIV-positive cats ( $n = 10$ , open boxes) (a), FeLV-positive cats ( $n = 5$ ; no boxes shown because of small numbers) (b), and FIV-FeLV-positive cats ( $n = 5$ ; no boxes shown because of small numbers) (c). \*,  $P < 0.05$  (Wilcoxon rank sum test).

explained by the known interfering effect of FeLV on erythrocyte progenitor cells (47, 63).

**Lymphocyte subsets.** In FIV-positive cats,  $CD4^+/CD8^+$  ratios were lowered significantly mainly in long-term infection (Fig. 5). The lowered  $CD4^+/CD8^+$  ratio was predominantly due to lowered  $CD4^+$  counts (Fig. 4). Our finding that the lowest ratio was seen in the FIV-FeLV-positive cats (Fig. 5) is in agreement with other reports (50). In FeLV infection, immunosuppression may be explained by marked transient lymphopenia and neutropenia which take place during the primary phase of the infection (49) and the immunosuppressive effect of the protein p15E during chronic FeLV infection (25, 40, 67). In our study, we found that both  $CD4^+$  (Fig. 4) and to a lesser degree also  $CD8^+$  lymphocytes were decreased in long-term FeLV infection compared with that in noninfected con-

TABLE 2. Differences in biochemical parameters in FIV-positive cats versus controls

Parameter	Mo <sup>a</sup>	Value <sup>b</sup> in:						P <sup>c</sup>
		Controls			FIV positive cats			
		5%	50%	95%	5%	50%	95%	
Cholesterol (mmol/liter)	56	7.7	9.4	12.7	4.7	8.1	9.3	0.0453
Glucose (mmol/liter)	9	3.2	3.7	4.5	3.5	4.4	4.9	0.0535
	19	4.0	4.3	4.7	4.2	4.7	5.3	0.0439
	43	4.8	5.1	5.4	4.7	5.8	8.5	0.0403
	56	4.8	5.3	5.6	5.3	6.3	7.2	0.0052
	64	4.3	4.7	5.0	4.4	5.2	5.9	0.0594
	75	4.6	4.8	5.0	4.6	5.1	5.3	0.0234
Lipase (U/liter)	9	7.5	11	26.7	16	24	32.2	0.0369
Phosphorus (mmol/liter)	43	1.1	1.2	1.4	1.2	1.4	1.5	0.0171
	56	1.3	1.4	1.6	1.5	1.6	1.9	0.0017
	64	1.1	1.3	1.4	1.2	1.3	1.6	0.0838
Protein (g/liter)	19	62	68	76	69	73	79	0.0376
	43	62	64	69	64	66	70	0.0722
	56	66	69	73	70	72	80	0.0262
	73	62	66	70	66	68	75	0.0482
	79	63	65	69	66	69	73	0.0215
	Sodium (mmol/liter)	43	161	162	165	162	165	168
Triglyceride (mmol/liter)	56	0.4	0.4	0.7	0.4	0.5	1.6	0.0361
	73	0.2	0.4	0.5	0.4	0.6	0.9	0.0102
	Urea (mmol/liter)	19	7.7	9.2	10.7	8.8	10.4	13.0
	30	8.3	9.0	10.7	9.0	10.2	11.7	0.0410

<sup>a</sup> Months after FIV infection took place in the experiment.

<sup>b</sup> Values are given for 5% quantile, median, and 95% quantile.

<sup>c</sup> By Wilcoxon rank sum test.

trols. Therefore, the  $CD4^+/CD8^+$  ratio was not significantly changed in these cats (Fig. 5). Similar observations were made for short-term FeLV infection (53, 66). Based on our findings, we postulate that in addition to the short-term depression of lymphocyte counts and the malfunctioning of T lymphocytes partly attributed to p15E (25, 40), a decrease of mainly  $CD4^+$  lymphocytes is an important factor in long-term FeLV-induced immunosuppression.

**Clinical chemistry.** The most consistent changes in biochemical parameters in FIV-infected cats were increased glucose and protein values and decreased cholesterol levels already early in infection and increased triglyceride values later in FIV infection (Table 2). Elevated concentrations of glucose (Fig. 7) may reflect changes in the energy metabolism of FIV-infected cats. Whether elevated glucose levels in our FIV-infected cats may reflect a hypermetabolic state (11, 70) associated with subclinical hypercortisolemia as is commonly found in human immunodeficiency virus (HIV)-infected humans (11) remains to be elucidated. Stress as a cause of hyperglycemia can be ruled out since our cats were highly accustomed to the caretakers and veterinarians and were slightly sedated for the blood collection. In addition, living conditions and blood collection procedures were identical for all cats.

The elevated concentrations of serum protein in FIV-infected cats (Table 2 and Fig. 6) could readily be explained by the increased gamma globulin levels found in these animals

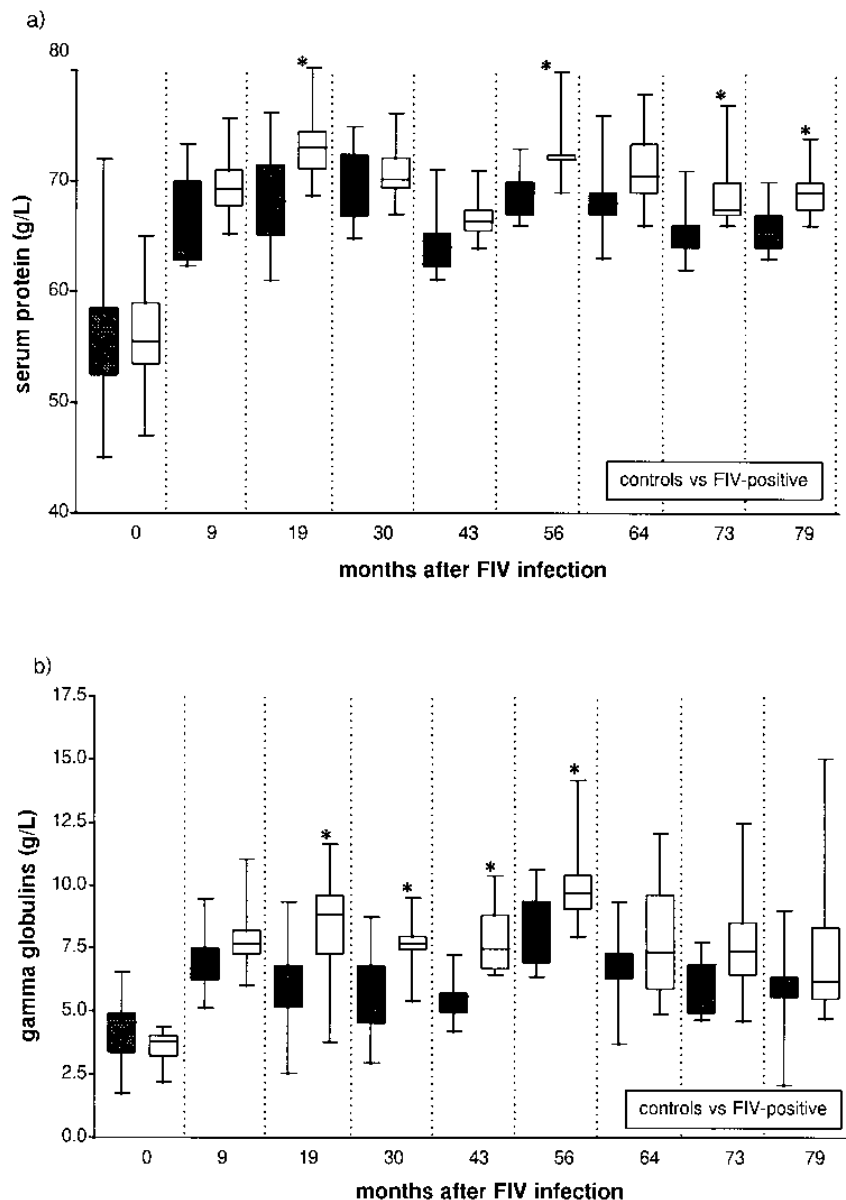


FIG. 6. Box plots of serum protein levels (a) and absolute gamma globulins (b) in controls ( $n = 10$ ; shaded boxes) and FIV-positive cats ( $n = 10$ ; open boxes). \*,  $P < 0.05$  (Wilcoxon rank sum test).

(Fig. 6). Hypergammaglobulinemia is associated with FIV infection (58, 62, 64), may reflect a polyclonal activation of the humoral immune response (1, 28), and is also a consistent finding in HIV-infected humans (3, 26, 30, 56). It is interesting to note that the statistically significant differences in the gamma globulins disappeared after month 56 (Fig. 6). This was due to an increased variance of the gamma globulin fraction in the FIV-positive cats and may reflect the beginning of a decreased B-cell activation and, with it, an additional step towards immune suppression in some of the FIV-positive cats.

The observation that cholesterol levels were decreased and triglyceride values were increased in FIV-positive cats is in striking agreement with the situation in HIV infection (13, 43, 60). Alterations in lipid metabolism are known to occur during a variety of infections (2, 4, 27, 55) and may reflect the stage and severity of disease (5). In HIV-infected humans, lipid

metabolism is known to be disturbed already in the asymptomatic phase (15). Cytokines such as tumor necrosis factor alpha, interleukin-1, and interferons are discussed as mediators of these metabolic alterations (12, 13). Alpha interferon levels are correlated with triglyceride levels, prolonged triglyceride clearance time (13), and increased de novo hepatic lipogenesis (15). The cytokine profile of our cats remains to be investigated. Regardless of the cause, these alterations are of particular interest in the context of the immune status of the infected individuals since they may influence cellular functions, for example, through changes in lipid membrane composition: the function of both the humoral and cellular immune systems may be impaired by these changes (9, 43, 60). Changes in serum lipid levels may therefore further contribute to the pathogenesis of AIDS in humans and the AIDS-like disease in FIV-infected cats.

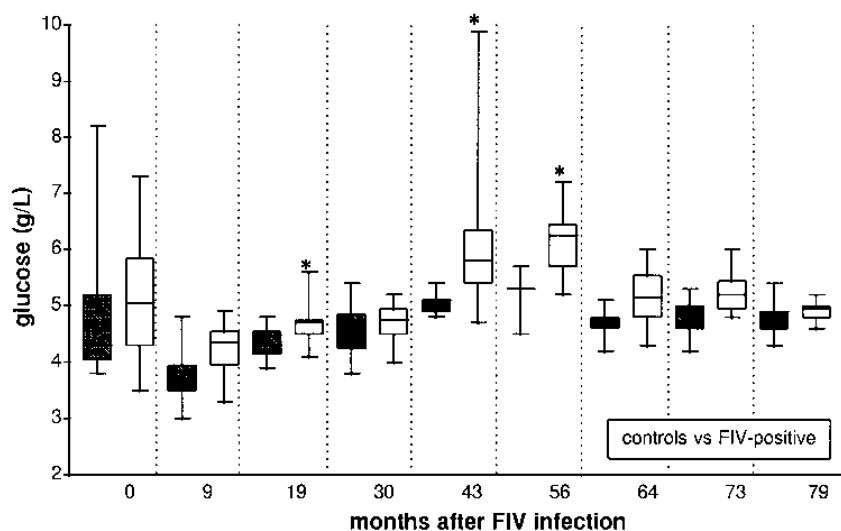


FIG. 7. Box plots of glucose values in controls ( $n = 10$ ; shaded boxes) and FIV-positive cats ( $n = 10$ ; open boxes). \*,  $P < 0.05$  (Wilcoxon rank sum test).

In addition, analysis of biochemical parameters revealed systematically higher urea levels in the FIV-positive (Table 2), the FeLV-positive (Table 3), and FIV-FeLV-positive cats compared with that in controls. Furthermore, phosphorus was significantly increased in FIV-positive cats after the feed change towards more canned food. There are several physiological factors which can influence the blood phosphorus values such as phosphorus intake or age of the animal or hemolysis of the sample. Since blood samples were always taken with utmost care, thereby minimizing hemolysis, and centrifugation and collection of sera were accomplished at the same time for all samples, elevated phosphorus values in FIV-positive animals may not be attributed to handling errors. Furthermore, age-dependent differences in phosphorus levels can be ruled out in this study because the animals of the four groups were age matched and, also, an influence of the food can be excluded as mentioned above. Elevated urea and phosphorus levels in FIV-positive cats may therefore be associated with a mild kidney dysfunction accompanied by a decreased glomerular filtration rate. A closer look at creatinine values of FIV-infected cats also revealed slightly higher mean creatinine levels in FIV-positive cats beginning 43 months after FIV infection (data not shown). These subclinical changes in kidney function may, with prolonged duration of FIV infection, also reflect pathologic

alterations in the kidney. In naturally FIV-infected cats, kidney abnormalities such as mesangial widening and segmental diffuse glomerulosclerosis or glomerulonephritis were reported (51, 54). Immune complexes deposited in the glomerula of FIV-infected cats may contribute to a decreased glomerular filtration rate (52). Moreover, elevated urea levels in FeLV-positive and FIV-FeLV-positive animals may be explained by a mild kidney dysfunction since FeLV infection is known to lead to glomerulonephritis (7, 21) partly due to deposition of circulating immune complexes (8, 61).

Sodium values were elevated in FIV-infected cats at some time points of the observation period (Table 2). No explanation can be given for this observation, since sodium intake levels were similar for all four groups, water was available ad libitum, room temperature was constant, and no dehydration was found upon clinical examination. A slight elevation of lipase levels was found in FIV-positive cats compared with that in controls beginning already 9 months after infection (Table 2). Pancreatitis was observed in some HIV-infected patients (44) but was at least partly attributed to infections with other pathogens such as cytomegalovirus or secondary effects of antiretroviral treatment (44, 71). Markedly elevated lipase values are associated with pancreatitis in the cat as well. Since pancreatitis is not known to occur in FIV infection and we did not have any clinical or laboratory evidence of pancreatitis, the slightly increased values of lipase found in the FIV-positive cats may be indicative of beginning kidney dysfunction and therefore may be in agreement with the findings described above.

In FeLV-infected animals, elevated AST levels were observed (Table 3). This phenomenon may be explained by microhemolytic processes not detected by the naked eye possibly caused by adsorption of FeLV to the surface of erythrocytes (45).

It is interesting to note that although the differences in biochemical parameters between FIV-positive and -negative cats were significant, most of the values still lay within the reference ranges evaluated for field cats in our laboratory. These differences were detected by comparing the parameters under study with those of a control population strictly matched for age and living conditions. This underlines the importance of appropriate control populations.

TABLE 3. Differences in biochemical parameters in FeLV-positive cats versus controls

Parameter	Mo <sup>a</sup>	Value <sup>b</sup> in:						P <sup>c</sup>
		Controls			FeLV-positive cats			
		5%	50%	95%	5%	50%	95%	
AST (U/liter)	30	15.5	19.5	23.7	21.6	25.0	50.2	0.0165
	43	16.2	21.0	26.4	21.6	28.0	39.5	0.0524
	56	18.0	20.0	24.6	23.0	23.0	41.9	0.0926
	73	16.8	20.0	23.6	36.1	36.5	37.0	0.0432
Urea (mmol/liter)	19	7.7	9.2	10.7	11.3	11.6	12.2	0.0048
	73	10.2	11.9	14.6	15.3	16.8	18.3	0.0451

<sup>a</sup> Months after FIV infection took place in the experiment.

<sup>b</sup> Values are given for 5% quantile, median, and 95% quantile.

<sup>c</sup> By Wilcoxon rank sum test.

In conclusion, the important parameters of disease progression were glucose, protein, gamma globulins, urea, cholesterol, and CD4<sup>+</sup> counts in FIV infection and MCV, MCH, urea, AST, and CD4<sup>+</sup> counts in FeLV infection. Monitoring of these parameters may prove useful for the evaluation of candidate FIV vaccines and antiretroviral drugs in cats. The many parallels between laboratory parameters in FIV and HIV infection further support the importance of FIV as a model for HIV (6, 10, 22).

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