Panleukopenia-like syndrome of FeLV caused by co-infection with FeLV and feline panleukopenia virus

H. Lutz*a,*, I. Castelli*a, F. Ehrenspergera, A. Pospisichlb, M. Rosskopf*b, G. Siegd, M. Grobe, S. Martinoid

aDepartment of Veterinary Medicine, University of Zurich, Zurich, Switzerland
bDepartment of Veterinary Pathology, University of Zurich, Zurich, Switzerland
cDepartment of Virology, University of Zurich, Zurich, Switzerland
dInstitute of Clinical Microbiology and Immunology, St. Gallen, Switzerland
eVeterinaria AG, Zurich, Switzerland
fSmithKline Beecham, Animal Health, Lincoln, NE, USA

Abstract

To study the effect of interferon on feline leukemia virus (FeLV) infection, 30 specific pathogen free (SPF) cats were infected with the apathogenic FeLV A Glasgow. Unexpectedly, between 5 and 8 weeks after FeLV infection, all 19 cats with persistent FeLV infection but not the FeLV-negative cats died from a panleukopenia-like syndrome. No feline panleukopenia virus (FPLV) antigen was found in feces by latex agglutination, enzyme-linked immunosorbent assay (ELISA) or immunoelectron microscopy. No enteropathogenic bacteria were found. Histopathology revealed changes resembling those of FPLV infection such as destruction of crypts and pancytopenia of bone marrow. Neither clinical signs nor seroconversion to FPLV could be induced by transmitting intestinal extracts to two SPF cats. However, FPLV antigen was demonstrated by immunofluorescence assay in intestinal cryostat sections of diseased animals. FPLV could also be demonstrated in intestinal extracts by immunoelectron microscopy, by latex agglutination and ELISA after anti-FPLV antibodies were removed from immune-complexed FPLV by ultracentrifugation over a CsCl gradient at pH 2.0. From these experiments it was concluded that the panleukopenia-like syndrome of FeLV may not be caused by FeLV alone but at least in some cases by co-infection with FeLV and FPLV. In addition, some form of 'cooperation' between FeLV and FPLV must be postulated because neither virus alone induced symptoms.

Abbreviations

CPV, canine parvovirus; ELISA, enzyme-linked immunosorbent assay; FeLV, feline leukemia virus; FPLV, feline panleukopenia virus; SPF, specified pathogen free.

* Corresponding author.
1. Introduction

The diseases caused by feline leukemia virus (FeLV) infection include, among others, lympho- and myeloproliferative diseases, anemia, immunosuppression and the so-called panleukopenia-like syndrome (Hardy et al., 1976). The panleukopenia-like syndrome of FeLV infection which was first described by Hardy et al. (1976) is found in FeLV-viremic cats vaccinated against feline panleukopenia virus (FPLV). The clinical signs of panleukopenia-like syndrome of FeLV are similar to those of FPLV infection and include anorexia, weight loss, vomiting and diarrhea. The leukopenia with values of 300–3000 leukocytes μl⁻¹ which is found in these cats often in conjunction with mild anemia is considered to be a consequence of myeloid destruction. In cats with panleukopenia-like syndrome, histologic examination of the small intestine reveals enteropathy characterized by atrophy of the villi and hemorrhagic and necrotizing mesenteric and regional lymph nodes. While FPLV replicates primarily in the crypts of the small intestine, in chronic cases of FPLV infection apical epithelial cells may also be involved.

In the present study in which we originally wanted to investigate the effect of human recombinant interferon alpha on FeLV infection we observed a panleukopenia-like syndrome in those cats that became persistently viremic with FeLV. FeLV-negative cats were not affected. Although FPLV was not readily demonstrated in feces, we suspected that FPLV was etiologically involved in the disease. We here report on the experiments that were done in order to demonstrate involvement of FPLV in these cases.

2. Materials and methods

2.1. Experimental design and chronology

Thirty specified pathogen free (SPF) cats were kept in a large room which was subdivided into three compartments by wire nets. In order to study the effect of a recombinant interferon alpha on FeLV infection, ten cats (Group A) received continuing interferon alpha treatment before and after experimental FeLV infection. Ten cats (Group B) received continuing interferon alpha treatment after experimental FeLV infection and ten cats (Group C) instead of interferon received a placebo whenever cats in Group A were treated with interferon. All 19 cats which became persistently FeLV-infected after experimental inoculation died between 5 and 8 weeks after experimental FeLV infection. The deaths occurred in all three groups at a similar incidence. Ten FeLV-negative cats survived; one FeLV-negative cat died much later than the 19 FeLV-positive animals in Week 11. All cats were necropsied, organ material was stored in formalin and also frozen at −30°C and the experiment was terminated. The rooms were carefully cleaned with high pressure steam and detergents and sterilized by formalin vapor. In a second experiment, 20 cats were transferred into these same rooms from another location. These 20 cats had been used for another study in which the immune reaction to rabies vaccination in FeLV-positive cats was studied (Franchini, 1990). Of these 20 cats, 15 had been experimentally infected with FeLV and five served as FeLV-negative controls. Between 2 and 3 weeks after the 20 cats had been transferred to the new location, again the FeLV-positive cats started to become sick and died or had to be
euthanized. In all cases the clinical symptoms included elevated body temperature, anorexia, exsiccosis and in most cases bloody diarrhea, vomiting, salivation, pale mucous membranes, normocytic, normochromic anemia and leukopenia. The 20 cats of Experiment 2 were part of a group of 30 cats. The remaining ten cats did not show clinical signs and survived their mates at another location where they were used for another study. All 50 cats had originally been infected with an FeLV preparation originating from the same tube. From these observations it was concluded that the disease symptoms could not have been caused by the FeLV but must be due to an additional factor related with the room, possibly to FPLV. Examination of all the diarrhea samples for presence of FPLV were negative. However, histopathology revealed a picture in agreement with FPLV infection. In order to demonstrate FPLV, pooled organ extracts were prepared from a number of diseased animals and transmitted into two SPF cats kept at the University of Zurich. When no clinical signs or seroconversion to FPLV was observed, this transmission experiment was repeated after the two cats had been pretreated with a high dose of corticosteroids to induce immunosuppression. When again this transmission experiment failed, we suspected that the parvovirus expected to be present in the diseased cats would be immune-complexed. Therefore, intestinal extracts were spun over a sucrose layer of pH 2.0 into a CsCl gradient in the ultracentrifuge. FPLV was found at the buoyant density of parvovirus.

2.2. Cats

2.2.1. Experiment 1

SPF cats were obtained from Ciba-Geigy (Werk Stein, Switzerland) at the age of 10–12 weeks. They had been vaccinated two times with a commercially available inactivated FPLV, herpes- and calcivirus vaccine. The animals were kept in a large room which was subdivided by wire nets into three compartments.

2.2.2. Experiment 2

Twenty cats 19 months of age were transferred to the same room in which cats of Experiment 1 had been housed. Fifteen of these cats had been experimentally infected with FeLV A 16 months before; 13 had become persistently viremic. Five cats served as negative controls.

2.3. FeLV and experimental infection

FeLV A Glasgow, kindly provided by Prof. O. Jarrett, Glasgow, was used for infection in a dose of $10^6$ focus-forming units in 1 ml of cell culture medium. Infection was done by instillation of 0.3 ml into each nostril and 0.4 ml into the mouth.

2.4. Clinical examination and collection of blood samples

The cats were checked daily for behavioral changes. If they showed signs of disease they were examined clinically. Blood samples were collected two times a week under superficial sedation using 5 mg Zoletil (Virbac Laboratories, Nice, France) per kg body weight.
2.5. Hematology and clinical pathology

Hematology measurements were done by an electronic cell counter (Contraves Analyzer 820; Contraves, Zurich, Switzerland); clinical chemistry parameters were determined by a Cobas Mira Analyzer (Roche Diagnostica, Basel, Switzerland).

2.6. Necropsies and histology

Necropsies were performed at the Department of Veterinary Pathology, University of Zurich, under routine procedures. Organ material was fixed in 10% (v/v) phosphate-buffered formalin solution and processed for microscopy using routine methods.

2.7. Diagnosis of FeLV infection

Plasma or serum samples were analyzed for FeLV p27 by an enzyme-linked immunosorbent assay (ELISA) procedure (Lutz et al., 1983). In addition, blood smears were subjected to an indirect immune peroxidase assay using a combination of three monoclonal antibodies (one specific for p27, two specific for gp70) according to conditions described elsewhere (Schniewind, 1982).

2.8. Demonstration of anti-FPLV antibodies

Serum samples collected from several cats of Experiment 1 were tested by the hemagglutination inhibition test described by Carmichael et al. (1980). For the detection of parovirus antibodies in the serum of cats experimentally infected with organ extract canine parovirus (CPV) was used to establish an ELISA. CPV grown in MDCK cell culture was purified by CsCl sucrose gradient purification in the ultracentrifuge as described by Castelli (1993). CPV antigen was used to coat micro-ELISA wells (100 ng per well) under conditions described elsewhere (Lutz et al., 1980). Rabbit anti-cat IgG conjugated to horseradish peroxidase (Milan Diagnostics, La Roche, Switzerland) was used in a dilution of 1:1000. With each ELISA plate four wells each of a positive and a negative control serum were included to standardize the optical density (OD) readings of the samples. The mean OD values of the two control sera were defined as 100% and 0%, respectively.

2.9. Demonstration of FPLV and FeLV in tissues

To demonstrate FeLV and FPLV in tissues, 5 μm cryostat sections were fixed in acetone for 5 min at 22°C. Sections were incubated by a mixture of monoclonal antibodies to FeLV (one against p27, two against gp70) or a monoclonal antibody to FPLV for 1 hour at 37°C. This anti-parovirus antibody was a generous gift from Dr. A. Aubert, Virbac Laboratories, Carros, France. After washing in phosphate-buffered saline (PBS), bound antibody was visualized by incubating the sections with goat anti-mouse IgG conjugated to fluorescein isothiocyanate (Milan Diagnostics). Before microscopy, the sections were counterstained for 4–5 s in Evans blue (0.1 g l⁻¹ in PBS) followed by a three-fold washing in PBS.
2.10. Production of an anti-FPLV antiserum

Purified CPV was inactivated by incubation for 48 h in 0.1% (v/v) β-propiolactone. A goat was immunized with 20 μg CPV antigen in 1 ml of PBS and 1 ml of incomplete Freund’s adjuvant followed by six booster immunizations 3 weeks apart under identical conditions.

2.11. In vitro experiments to isolate FPLV

A total of 4 g of different organs (stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, spleen, bone marrow, tonsils) was obtained from sick cats of Experiment 2 and frozen in liquid nitrogen. The material was homogenized together with 5 ml of PBS in a Virtis homogenizer (10,000 r.p.m. for 3 X 1 min) on ice. The suspension was frozen and thawed three times, pelleted (20 min, 5000g) and passed through a Millipore filter (0.2 μm; Millipore, Bassersdorf, Switzerland). Aliquots of 100 μl of the tissue homogenates, CPV (as positive control) and PBS (negative control) were used to inoculate CrFK cell cultures. Ten days after a cytopathogenic effect had appeared in the positive CPV control, the cultures were fixed in methanol for 5 min and analyzed for parvovirus and FeLV by immunofluorescence and immunoperoxidase methods as described above.

2.12. In vivo transmission experiments

Two SPF cats 12 weeks of age which had not been vaccinated against feline parvovirus were injected intraperitoneally with 1 ml of tissue homogenate prepared as described above. The cats were clinically examined daily, temperature was measured every second day and samples of blood and feces were collected every second day until Day 16 after infection. Blood samples were analyzed for complete blood cell counts including differentiation, for anti-parvovirus antibodies and for FeLV p27. Samples of feces were analyzed for FPLV antigen by a latex agglutination test (ANI Parvotest; Sirebio, Paris, France). When no signs of FPLV infection were found, the two cats were treated by corticosteroids to induce immunosuppression as described by Post and Warren (1980). When the lymphocyte counts reached a minimum 2 weeks after steroid treatment, the cats were again infected using tissue homogenates as described above. Over a period of 11 weeks a total of 12 blood and feces samples was obtained.

2.13. Demonstration of parvovirus antigen in feces and organ extracts

For the demonstration of FPLV in feces and organ extract a commercial ELISA (Diasystems® Canine Parvo; Tech America, Colorado, USA) and a latex agglutination test (ANI Parvotest; Sirebio) were used. In addition, these materials were also analyzed for FPLV by immune electron microscopy under conditions described elsewhere (Zanoni et al., 1984).

2.14. Demonstration of FPLV by CsCl gradient centrifugation

When the in vitro and in vivo transmission experiments did not reveal infectious parvovirus it was suspected that FPLV would be present in the form of immune complexes. To
disrupt these immune complexes, 10 ml of organ extracts were loaded on the top of a gradient in 38 ml polyallomer tubes (Beckman Instruments, Palo Alto, CA, USA). The gradient consisted of 5 ml of CsCl in TES buffer (50 mmol Tris-HCl, pH 8, 1 mmol EDTA, 0.1% (v/v) sodium N-lauroylsarcosine) at a density of 1.5 g ml\(^{-1}\). The second layer consisted of 4 ml of CsCl in TES buffer (density 1.363 g ml\(^{-1}\)); the third layer consisted of 4 ml of CsCl in TES buffer (density 1.3 g ml\(^{-1}\)) and the fourth layer consisted of 6 ml of 30% sucrose solution in acetate buffer (pH 2.0). The gradients were centrifuged in a SW-27 Rotor (Beckman Instruments, Palo Alto, CA, USA) at 25 000 r.p.m. for 16 h at 4 °C. The gradients were then eluted from bottom to top and fractioned into 0.5 ml aliquots which were analyzed for FPLV antigen and for density by an Atago refractometer (Model No. 302, ABBA Refractometer, Japan). The presence of FPLV antigen was revealed by ELISA. Aliquots of 10 µl of each fraction were used to coat ELISA wells. Antigen was detected by goat anti-parvovirus serum (1:300) and subsequent incubation with rabbit anti-goat peroxidase conjugate (Milan Diagnostics) at a dilution of 1:1000.

3. Results

3.1. Clinical course, laboratory results and necropsy findings of cats

3.1.1. Experiment 1

Of the 30 cats of Experiment 1, 19 became persistently viremic. These 19 animals died between 5 and 8 weeks after FeLV infection. One additional cat which overcame primary FeLV infection died 15 weeks after FeLV inoculation. The clinical symptoms included pyrexia, anorexia, dehydration, diarrhea (sometimes bloody), vomiting, salivation, pale mucous membranes, normocytic and normochromic anemia and absolute leukopenia. In most cases the clinical course lasted 2–3 days, in a few cases up to 10 days. Without exception, FPLV was not detected by ELISA, latex agglutination or electron microscopy in feces and organ extracts. No Salmonella sp., Campylobacter jejuni/coli or Clostridium perfringens was detected. Necropsy revealed catarrhal to necrotizing enteropathy in combination with crypt atrophy, occasionally intranuclear inclusion bodies in the ileum, sinus histiocytosis with erythrophagocytosis of mesenteric lymph nodes, hyperplasia of spleen follicles, degeneration of lymphatic tissue in Peyer's patches, acute hemorrhagic diathesis in the intestine, epicardium and peritoneum, and depletion of bone marrow. Five animals died in spite of treatment while 15 were euthanized for humane reasons. From the ten animals that had to be euthanized, serum samples collected 1 week before FeLV infection and in the week of death were subjected to the hemagglutination inhibition test for the demonstration of FPLV antibodies. Four of the ten cats had a four-fold increased parvovirus titer shortly before death while the other six cats had titer differences of a factor of 2 or lower.

3.1.2. Experiment 2

Of the 20 cats of Experiment 2 (13 of 15 previously infected cats had become persistently FeLV-positive, five served as negative controls) the 13 persistently FeLV-positive cats became acutely sick and had to be euthanized within 2–3 weeks after they had been
transferred to the new rooms. Clinical symptoms, laboratory results and necropsy findings were identical to those in Experiment 1. Again, no parvovirus was found by ELISA, latex agglutination and immune electron microscopy in bone marrow, feces and organ extracts of the 13 cats.

3.2. Detection of FPLV and FeLV in cryostat sections

Cryostat sections obtained from various samples of the small intestine, lymph nodes and bone marrow were analyzed for FPLV and FeLV antigen by immunofluorescence assay using monoclonal antibodies. FPLV and FeLV antigen was readily detected in most of the sections examined (Figs. 1(a) and 1(b) and Table 1).

3.3. Isolation of FPLV in cell cultures

In two of two CRFK cell cultures inoculated with organ extracts no parvovirus antigen was detected by immunofluorescence. In two of two positive controls parvovirus antigen was readily detected.
Table 1
Immunohistochemical demonstration of FPLV and FeLV antigen in different organ material collected from a total of 13 FeLV positive cats with clinical signs in Experiment 2

<table>
<thead>
<tr>
<th>Organ</th>
<th>FPLV antigen</th>
<th>FeLV antigen</th>
<th>Positive for FeLV and FPLV*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive/No. tested</td>
<td>No. positive/No. tested</td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Jejunum</td>
<td>7/8</td>
<td>3/8</td>
<td>2/8</td>
</tr>
<tr>
<td>Ileum</td>
<td>3/5</td>
<td>2/5</td>
<td>1/8</td>
</tr>
<tr>
<td>Peyer's plaque</td>
<td>0/2</td>
<td>1/2</td>
<td>0/8</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>3/9</td>
<td>9/9</td>
<td>3/9</td>
</tr>
<tr>
<td>Lymph node</td>
<td>1/14</td>
<td>14/14</td>
<td>1/14</td>
</tr>
</tbody>
</table>

*Sections were incubated with either anti-FeLV or anti-FPLV antibodies.

3.4. In vivo transmission experiments

No hematologic changes, no FPLV antigen and no rise in FPLV antibody titers were observed after the first and the second inoculation with organ extracts (Fig. 2). Immunosuppressive treatment was initiated on Day 39 after the first inoculation in one of the cats (Cat 460). Very low parvovirus antibodies developed in this animal which peaked on Day 52 and thereafter declined to values identical to those at the beginning of the experiment. The third inoculation which took place 14 days after starting immunosuppressive treatment (Day 55) did not lead to any antibody response (Fig. 2). As the antibody titers did not reach high levels and were not in temporal relationship with either of the inoculations, it was concluded that they were not specifically directed against parvovirus.

Fig. 2. Course of anti-FPLV antibodies in two cats inoculated with organ extracts. The extracts were obtained from pooled organs collected from cats in Experiment 2. Antibodies were measured by ELISA as described in the text.
Fig. 3. Demonstration of FPLV antigens in fractions collected from a CsCl gradient. Different material was homogenized as described in the text and separated by ultracentrifugation. Aliquots of 10 µl of the individual fractions were used to coat micro-ELISA wells. Gradient 1: MDCK cells infected with canine parvovirus as positive control. Gradient 2: organ extract collected from ten cats of Experiment 2. Gradient 3: organ extract obtained from one SPF cat. Gradient 4: distilled water. Gradients 3 and 4 served as negative control. All fractions collected from Gradient 1 tested positive, the highest activity being present in Fraction 14 (density of 1.374 g ml⁻¹). The extract from cats of Experiment 2 (Gradient 2) showed increased optical density in Fractions 14-16.

3.5. Detection of FPLV in CsCl gradients after disruption of the immune complexes

In pooled organ extracts collected from ten cats of Experiment 2, parvovirus antigen was detected in Fractions 14, 15 and 16 of the CsCl gradient by ELISA (Fig. 3) and by immune electron microscopy (Fig. 4). No antigens were detected in organ extracts obtained from small intestine, lymph nodes and bone marrow collected from an SPF cat which was not part of the experiment (Fig. 3). From these experiments it was concluded that FPLV was

Fig. 4. Immune electron microscopy of viral particles present in Fraction 14 of Gradient 2 shown in Fig. 3. The photograph reveals typical FPLV morphology. The length of the bar corresponds to 100 nm.
indeed present in the extracts but was not detected by the different immunological tests, probably because the virus was coated by feline immunoglobulins.

4. Discussion

The present paper deals with an unusual clinical outcome of experimental FeLV infection in cats and the clinical course, hematological, serological and pathological findings are described. Only cats were affected which developed persistent FeLV viremia. Cats in which no persistent FeLV viremia occurred and the non-infected controls were not affected. Clinical signs, hematology and necropsy findings indicated that the disease fulfilled the criteria of the panleukopenia-like syndrome. Panleukopenia-like syndrome of FeLV is a condition of FeLV viremic cats resembling that of FPLV infection. Several groups have described the panleukopenia-like syndrome of FeLV (Cotter et al., 1975; Hardy et al., 1976; Rojko et al., 1979; Reinacher and Theilen, 1987; Hoover et al., 1987). Many of the authors suspected FPLV to be etiologically involved but failed to demonstrate FPLV antigen (Reinacher and Theilen, 1987; Hoover et al., 1987). In our study we did not find FPLV until after immune complexes were dissociated.

Although we could not directly prove the involvement of FPLV by transmission experiments, several arguments support the involvement of FPLV. (1) The clinical signs and the pathological findings cannot be explained by FeLV infection alone. The virus used to infect the animals of Experiment 1 originated from the same vial which was used to infect 20 cats in another experiment several months before (Franchini, 1990). At the time of the present study those cats were still FeLV-infected but without exception were completely healthy. The cats of the present study originated from the same breeder as those used by Franchini (1990). With the exception of a single animal, none of the 11 FeLV-negative cats became sick. (2) In the second experiment 20 cats of the study of Franchini were transferred to the same rooms in which the animals of the first experiment had been kept. Although these rooms had been disinfected, again the 13 FeLV-positive cats died within a period of 2–3 weeks after they had been brought to the new rooms. The FeLV-negative animals, again, did not become sick. At the same time the remaining ten cats of the study of Franchini (1990) were moved to rooms at the University of Zurich and survived the experiments described here for many months without ever showing clinical signs (Franchini et al., 1990). The fact that the putative infectious agent survived disinfection of the rooms indicated that the agent must have been highly resistant to disinfection. Parvoviruses are known to have a high tenacity. (3) Clinical signs, hematology results and findings of necropsy and histology of the euthanized cats in both experiments were typical for FPLV infection. (4) Demonstration of parvovirus antigen in the crypt cells of the small intestine with the aid of a monoclonal antibody specific for FPLV can be considered a strong argument for an involvement of FPLV in the etiology of the disease. (5) The antigen found in the organ extracts after centrifugation through a sucrose cushion of pH 2.0 into a CsCl gradient had several properties which are typical for FPLV: the density of 1.374 g ml⁻¹ is typical for parvoviruses; the immunologic cross-reactivity of the antigen of Fractions 14, 15 and 16 by latex agglutination, ELISA and by immune electron microscopy is strongly suggestive for FPLV; the morphology of the agent detected by immune electron microscopy in Fraction
14 is also typical for FPLV. It is very unlikely that the parvovirus described here originated from the FeLV inoculum used to infect the cats of Experiment 1. This can be concluded from the fact that the 15 cats infected by Franchini (1990) received the very same FeLV preparation. They remained completely healthy until they were transferred to the same rooms where the first experiment was conducted.

There are also several arguments which contradict the involvement of FPLV. (1) The rooms where the two experiments were conducted were designed to carry out infectious experiments. Veterinarians and animal caretakers with access to these rooms were not allowed to have contacts with cats and dogs and had to undergo showering whenever they entered the rooms. (2) All cats had been vaccinated at least two times against FPLV before entering the experiment. (3) Detection of FPLV in feces and in untreated organ extracts was not successful, either by ELISA, or by latex agglutination or immune electron microscopy. (4) Transmission of a putative FPLV was unsuccessful in vitro and in vivo. These latter four points speak against a co-infection of FeLV and FPLV but, however, do not rule it out. The following explanations can be offered: (1) Because of their high tenacity, parvoviruses are known to be present almost ubiquitously. It can therefore be imagined that they could have been brought into the room in spite of SPF conditions. (2) FeLV infection leads to immunosuppression. The fact that only FeLV-positive cats were affected can explain that vaccination-induced immunity against FPLV was compromised by FeLV infection. (3) Failure to demonstrate FPLV antigen by the three immunological approaches can be explained by coating of FPLV by the cats' immunoglobulins. When the antibodies were removed by centrifugation through a sucrose solution of low pH, FPLV was readily detected with each of the three techniques. (4) The fact that the in vitro cell culture isolation and the in vivo transmission experiments were not successful can also be explained by coating of FPLV by autologous cat immunoglobulins and/or by the low virus dose present in the inoculum. Although FeLV-mediated immunosuppression could readily explain the FPLV infection, another mechanism has also to be considered. The genus Dependovirus of the Parvovirus family includes viruses which depend for their replication on helper functions from other viruses such as adeno-, herpes- or vaccinia viruses (Siegl, 1976). It could be imagined that the parvovirus found here could profit from certain helper functions provided by the FeLV. Further experiments would be necessary to study this possibility.

Originally it was the goal of Experiment 1 to study the effect of an interferon alpha on the course of FeLV infection. That the interferon treatment could be connected with the clinical signs has to be considered. This is especially the case because high doses of interferon have been described to cause immunosuppression (Johnson et al., 1975; Johnson and Baron, 1976; Kirschmann and Murasko, 1991; Take et al., 1992). That interferon could not have been of high importance can be concluded from the fact that in Experiment 1 the first cats that showed clinical signs were those that had received the placebo. In addition, cats of Group A, which had received interferon before FeLV was inoculated, survived distinctly longer than all other cats.

We concluded from these experiments that the clinical signs were caused by a co-infection with FeLV and FPLV. Whether and how frequent co-infection with FeLV and FPLV occurs in the field is unknown. We have reason to suspect that double infections are quite frequent because both viruses are relatively prevalent in the cat population. FeLV viremia can be found at a prevalence of between 6 and 18% (Hardy et al., 1977; Boller and Von Steiger.
In the early seventies FPLV infection was quite prevalent (Gillespie and Scott, 1973). Today, the exact prevalence of FPLV infection is unknown. As most cats are vaccinated against FPLV infection, serological studies cannot differentiate between vaccination and infection induced antibodies. It will be interesting to study the importance of FeLV and FPLV co-infection in the field by assays with which the cats' antibodies do not interfere such as polymerase chain reaction or in situ hybridization.

Acknowledgements

This project was supported by Ciba, Basel, Switzerland and by a grant from the Swiss Banking Corporation on behalf of a customer. We thank Dr. M. Reinacher, Department of Veterinary Pathology, University of Leipzig, for his support with immunoperoxidase methods. We are indebted to H. Reutimann for the preparation of the manuscript and E. Hug for the photographs.

References


