Experimental Transmission and Pathogenesis of Immunodeficiency Syndrome in Cats

By Edward A. Hoover, James I. Mullins, Sandra L. Quackenbush, and Peter W. Gasper

We describe the identification, experimental transmission, and pathogenesis of a naturally occurring powerfully immunosuppressive isolate of feline leukemia virus (designated here as FeLV-FAIDS) which induces fatal acquired immunodeficiency syndrome (AIDS) in 100% (25 of 25) of persistently viremic experimentally infected specific pathogen-free (SPF) cats after predictable survival periods ranging from <3 months (acute immunodeficiency syndrome) to > one year (chronic immunodeficiency syndrome), depending on the age of the cat at time of virus exposure. The pathogenesis of FeLV-FAIDS-induced feline immunodeficiency disease is characterized by: a prodromal period of largely asymptomatic viremia; progressive weight loss, lymphoid hypoplasia associated with viral replication in lymphoid follicles, lymphoid depletion associated with extinction of viral replication in lymphoid follicles, intractable diarrhea associated with necrosis of intestinal crypt epithelium, lymphopenia, suppressed lymphocyte blastogenesis, impaired cutaneous allograft rejection, hypogammaglobulinemia, and opportunistic infections such as bacterial respiratory disease and necrotizing stomatitis. The clinical onset of immunodeficiency syndrome correlates with the replication of a specific FeLV-FAIDS viral variant, detected principally as unintegrated viral DNA, in bone marrow, lymphoid tissues, and intestine. Two of seven cats with chronic immunodeficiency disease that survived >1 year after inoculation developed lymphoma affecting the marrow, intestine, spleen, and mesenteric nodes. Experimentally induced feline immunodeficiency syndrome, therefore, is a rapid and consistent in vivo model for prospective studies of the viral genetic determinants, pathogenesis, prevention, and therapy of retrovirus-induced immunodeficiency disease.

MATERIALS AND METHODS

Animals

The specific pathogen-free (SPF) cats used in the transmission studies were from a breeding colony of cesarian-derived SPF cats maintained in the Department of Pathology, Colorado State University. These animals are free of infection with and immunity to horizontally transmitted feline viruses, including FeLV. Age-matched, noninoculated, noncontact control SPF cats were obtained from the same source, for which there is a substantial database of normal age-adjusted hematologic data.

Virus, Inoculation, and Sample Collection

The original FeLV-FAIDS inoculum consisted of a 20% (wt/vol) viable-cell-free extract of thymic lymphoma tissue from an FeLV-infected pet cat in which the lymphoma had regrown after regression induced by radiotherapy. In most inoculation experiments, virus was administered intraperitoneally (IP); in two series of inoculations, the
in tranasal route was used (both routes induced infection and disease) (Table 1). Inocula for serial passage experiments were prepared as 20% (wt/vol) extracts of bone marrow, spleen, or lymph node from animals that had developed fatal immunodeficiency disease in the first virus passage (Table 1). All inocula contained between 1 and 2 x 10^6 focus forming units (fu) of infectious FeLV.

One group of two weanling animals was inoculated with virus harvested from a feline fibroblast cell line inoculated with the original stock of FeLV-FAIDS. Likewise, FeLV-FAIDS was characterized morphologically by electronmicroscopic examination of glutaraldehyde-fixed, osmium tetroxide- postfixed inoculated feline fibroblast cell cultures.

Blood samples were collected from each inoculated cat prior to infection and at weekly intervals for the first 6 weeks postinoculation and biweekly thereafter. Bone marrow samples were collected at various intervals (most often biweekly) by needle aspiration from the femur or humerus of inoculated cats. For all inoculation, blood collection, and bone marrow sampling procedures, animals were anesthetized with ketamine hydrochloride.

### Assays to Monitor FeLV-FAIDS Infection in Cats

#### Immunoassay for FeLV p27 group-specific antigen in blood cells and tissues.

FeLV-FAIDS group-specific antigen (gag gene-coded major viral structural protein p27) was detected by indirect immunofluorescence staining of methanol-fixed blood films, and on methanol-fixed paraffin-embedded tissues. The goat anti-FeLV p27 antiserum used was extensively absorbed with feline blood and bone marrow cells to eliminate non-specific reactivity due to natural anti-feline tissue antibody in goat or rabbit sera. The persistent presence of FeLV p27 antigen in blood cells (neutrophils, platelets, lymphocytes) correlates >95% of all instances with the presence of infectious virus in blood.

#### Enzyme-linked immunosorbent assay (ELISA) for FeLV antibody.

FeLV antibody was determined by an ELISA using sucrose density-gradient purified whole FeLV (KT isolated from the FL74 lymphoblastoid cell line) as antigen, feline test serum, and peroxidase-conjugated goat-anti-feline IgG as the secondary reagent (Miles Scientific, Naperville, IL).

#### Quantitation of infectious virus in inocula.

Infectious FeLV in tissue extracts was determined by the clone 81 focus induction assay described by Fischer et al. P Article-associated reverse transcriptase was also demonstrated as described for FeLV by Nicolson et al.

#### Detection of viral DNA in tissues.

FeLV-FAIDS integrated and unintegrated viral DNA was detected in tissues of infected cats by Southern blotting using KpnI endonuclease digestion and hybridization to an exogenous FeLV-specific DNA probe that identifies a 250-base pair (bp) sequence in the unique region of the long terminal repeat of exogenous feline retroviruses only, as detailed by Mullins et al. Enzyme-linked immunosorbent assay (ELISA) for FeLV antibody was determined by an ELISA using sucrose density-gradient purified whole FeLV (KT isolated from the FL74 lymphoblastoid cell line) as antigen, feline test serum, and peroxidase-conjugated goat-anti-feline IgG as the secondary reagent (Miles Scientific, Naperville, IL).

#### Hematologic assays.

Complete blood cell counts were performed at biweekly intervals (Coulter S-plus calibrator for feline blood). In some cats with terminal immunodeficiency disease, bone marrow cell clonogenic colony-forming assays of erythroid and granulocyte/macrophage progenitor cells were performed in methycellulose semisolid medium using techniques described by Abkowitz et al and modified by Gasper et al. Albumin and globulin classes in terminal sera from some cats were determined by electrophoresis using standard procedures.

### Assays to Monitor FeLV-FAIDS Infection in Cats

#### Mitogen-driven, feline T lymphocyte blastogenesis was determined as previously described originally by Cockerell et al and extended by Rojko et al for feline blood lymphocytes. Blastogenesis assays were performed by incubating Ficoll-metrizoite-separated blood mononuclear cells with either concanavalin A (1 μg/2 x 10^6 cells, a feline T cell mitogen) or pokeweed mitogen (10 μg/2 x 10^6 cells, a feline T and B cell mitogen). Net cell DNA-associated uptake of 3H thymidine in cells pulse-labeled for 18 hours before harvest with 0.5 μCi of 3H thymidine/2 x 10^6 cells was then quantitated by liquid scintillation.

#### Histologic Examination

Necropsies were performed and macroscopic lesions were recorded for all cats that died or were killed due to symptoms of severe immunodeficiency disease. Duplicate tissue sections were collected in absolute methanol for immunofluorescence and in either formalin or Bovi's fixative for conventional histologic section preparation. The following tissues from each of 20 animals were examined histopathologically: mandibular, parotid, mesenteric, and colonic lymph nodes, spleen, bone marrow, thymus, liver, kidney, adrenal, lung, heart, and small intestine (duodenum, jejunum, ileum) colon, urinary bladder, trachea, esophagus, thyroid, nostril, and brain.

### RESULTS

#### Virus Isolation and Characteristics

The lymphocytopathic FeLV we describe was identified by intrathymic inoculation of newborn kittens with an extract of thymic lymphoma tissue from an FeLV-infected pet cat in which the lymphoma had regrown after regression induced by radiotherapy. Five of six inoculated kittens died with severe lymphoid depletion and enteritis within 2 months postinoculation (Table 1). The viral isolate (designated here as FeLV-FAIDS) was identified as a feline leukemia retrovirus52 by the following criteria: type C morphology by electronmicroscopy; immunoreactive FeLV group-specific (gag) and envelope proteins as determined by immunofluorescence and immunoelectrophoresis, respectively; virus particle-associated reverse transcriptase activity53,54; and FeLV interference inducing activity characteristic of FeLV subgroup A and B viruses55-58 (performed in the laboratory of Drs Oswald Jarrett and David Onions, University of Glasgow, Glasgow, Scotland).

#### Experimental Transmission and Disease Induction

To determine whether the lymphocytopathic activity of the FeLV-FAIDS isolate was restricted to or dependent on infection during the neonatal period, we inoculated groups of SPF cats aged 8, 15, and 24 weeks with the original virus stock. We then performed two serial virus subpassages in 8-week-old SPF cats using marrow, spleen, and lymph node inocula containing between 1 and 2 x 10^5 (infected) fu of FeLV (Table 1).
Table 1. Experimental Transmission of FAIDS in Specific Pathogen-Free Cats

<table>
<thead>
<tr>
<th>Age of Cats at Inoculation</th>
<th>Route of Inoculation</th>
<th>Virus Passage Number</th>
<th>Cats Inoculated (n)</th>
<th>Cats With Persistent Viremia (n)</th>
<th>Cats With FAIDS (n)</th>
<th>Survival Time of Viremic Cats (Days)</th>
<th>Cats With Lymphopenia (n)</th>
<th>Cats With Suppressed Lymphocyte Blastogenesis (n)</th>
<th>Cats With Prolonged Skin Graft Rejection (n)</th>
<th>Clinical Signs</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day</td>
<td>Intrathymic</td>
<td>I</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>59 ± 3</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>Weight loss, diarrhea</td>
<td>Severe lymphoid depletion, necrotizing enterocolitis</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>Intraperitoneal</td>
<td>I, II, III</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>99 ± 59</td>
<td>11</td>
<td>5/5*</td>
<td>2/2†</td>
<td>Weight loss, thrush, diarrhea</td>
<td>Lymphoid hyperplasia, severe lymphoid depletion, necrotizing enterocolitis, stomatitis</td>
</tr>
<tr>
<td>15 Weeks</td>
<td>Oroesal</td>
<td>I</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>241 ± 166</td>
<td>6</td>
<td>2/2</td>
<td>ND</td>
<td>Diarrhea, severe chronic weight loss</td>
<td>Lymphoid hyperplasia, severe lymphoid depletion</td>
</tr>
<tr>
<td>24 Weeks</td>
<td>Oroesal</td>
<td>I</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>451 ± 84</td>
<td>3</td>
<td>3/3</td>
<td>2/3</td>
<td>Severe chronic weight loss, diarrhea, anemia</td>
<td>Lymphoid depletion, enterocolitis</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>35</td>
<td>25</td>
<td>25</td>
<td></td>
<td>25</td>
<td>10/10</td>
<td>4/6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cats were inoculated with between 1 and 2 × 10⁶ IU FCV-FAIDS as determined by clone 81 assay. [4]

*Number affected/total inoculated.
†Prolonged rejection = >16 days (p < 0.05 by Student’s t test) (mean for normal SPF cats 13.1 ± 1.5 days, n = 20). A single unrelated donor was used for all cats.
Twenty-five of 35 cats of various ages (71%) inoculated with FeLV-FAIDS developed persistent viremia within 2 to 4 weeks after inoculation. All the viremic cats (25 of 25) died of immunodeficiency disease from 25 to 524 days after inoculation (mean 171 ± 156 days) (Table 1). None of 10 cats that developed transient, regressive infection after virus inoculation developed any sign of disease after observation periods as long as 600 days. ELISA antibody titers against FeLV-FAIDS developed in both progressively and regressively infected cats; however, titers of 25 viremic cats destined to develop immunodeficiency syndrome declined to minimal levels (geometric mean 1:4.3 ± 3.6) 3 to >40 weeks before clinical disease developed. The terminal mean viral antibody titer of ten regressively infected cats was 1:58 ± 5.6.

The susceptibility of SPF cats to induction of persistent FeLV-FAIDS viremia, the latent period for clinical immunodeficiency disease induction, and the incubation period for disease induction all were related to age of cat at time of inoculation (Table 1); 85% of animals <8 weeks old at inoculation developed persistent viremia and survived for an average of 80 ± 58 days postinoculation (DPI). In contrast, 56% of cats >15 weeks old at inoculation developed persistent viremia and survived for an average of 311 ± 173 DPI. Therefore, relative to other characterized FeLV isolates so studied, FeLV-FAIDS exhibited considerable virulence and was capable of producing persistent viremia and disease in young adult cats after a single intranasal or intraperitoneal inoculation and without the concurrent administration of adrenal corticosteroids, a cofactor required to induce >50% incidence of progressive infection in adult cats with many FeLV isolates.

Clinical Courses of Disease

Two clinical courses of feline immunodeficiency syndrome were recognized. The first clinical course, acute or early onset immunodeficiency disease, was characterized by a survival period of <180 DPI (mean 86 ± 42; range 25 to 160) and developed in 72% (18 of 25) of all viremic cats and 94% (15 of 16) of viremic cats that were 8 weeks or less at inoculation. A short (2 to 10 weeks) asymptomatic period was followed by progressive lymphocytopenia, suppressed lymphocyte blastogenesis, failure to gain weight and/or progressive loss of weight leading to emaciation (Fig 1A), persistent diarrhea, and opportunistic infections including bacterial rhinitis, pneumonia, and necrotizing stomatitis (Fig 1B).

The second clinical course, chronic or late-onset feline AIDS, was characterized by a survival period of >180 DPI (mean 396 ± 113, 4.6 times that of cats with acute AIDS) and occurred in 28% (7 of 25) of all viremic cats but in 67% (6 of 9) of cats aged ≥15 weeks at inoculation. The chronic disease course was marked by a relatively long (6 months to >1 year) and largely asymptomatic period of viremia, subnormal weight gain, and progressive lymphocytopenia. This prodromal period was followed by an accelerated symptomatic phase characterized by relentless diarrhea and accelerated weight loss leading to terminal debilitation. Thus, the chronic clinical course of experimental feline AIDS more closely simulated the protracted natural evolution of most cases in immunodeficiency disease in humans.

Hematologic and Immunologic Features of FeLV-FAIDS Infection

The mean lymphocyte count for all symptomatic and presymptomatic FeLV-FAIDS inoculated cats over the course of all sampling periods postinoculation (2 to 67 weeks) was significantly below that of age-matched controls (Fig 2, Table 2). Lymphocyte mitogen-driven blastogenesis characterized was depressed in symptomatic cats as compared with uninoculated controls (Figs 2 and 3 and Table 2). Skin allograft rejection time was significantly prolonged in four of five symptomatic FeLV-FAIDS–infected cats vs normal controls (Table 2). Gamma-globulin levels in terminal sera from ten AIDS cats assayed were 44% of those of controls (Table 2). Thus, evidence for severe deficits in both T and B lymphocyte function were present in cats with immunodeficiency syndrome.

Nonlymphocytic hematologic deficits also were detected in FeLV-FAIDS–inoculated cats. Circulating granulocytes and monocytes in virus-inoculated cats were significantly less than controls over the course of all sampling periods (Table 2). Terminal hematocrits of inoculated cats also were less than controls and evidence of erythropoietic regeneration was absent (reticulocyte counts were <50,000 in infected cats vs >150,000 in controls). Clonogenic colony-forming assays of bone marrow cells from each of four cats with immunodeficiency syndrome tested revealed suppression of erythroid progenitor cells without discernible impairment of myeloid progenitor cell growth (Table 2).

Lesions

A consistent array of lesions was present in cats with acute feline AIDS. These were: (a) thymic atrophy in all (13 of 13) cats examined histologically (thymic remnants were detectable only by microscopy); (b) lymphoid depletion involving follicular and parafollicular lymphocytes in lymph nodes, spleen, and Peyer’s patches, resulting in ablation of entire follicles (9 of 13 animals) (Fig 1B and D); (c) follicular lymphoid hyperplasia in some cats (6 of 13) characterized by large, sometimes irregularly shaped follicles with prominent germinal centers comprised of lymphoblasts (Fig 1C); and (d) necrotizing enterocolitis (13 of 13) marked by necrosis and partial regeneration of the mucosal germinal epithelium, neutrophil and macrophage infiltration, and atrophy of mucosal villi (Fig 1F). The histopathogenesis of the lymphoid lesions in FeLV-FAIDS–infected cats, therefore, resembles that in human AIDS, ie, an apparent sequence of follicular lymphoid hyperplasia of variable duration followed by progressive follicular ablation and ultimately extensive parafollicular and medullary lymphoid depletion.

Lesions of lymphoid depletion in cats with chronic course of disease were more pronounced than in animals with the acute syndrome. Bone marrow cellularity in terminal immunodeficiency cats was significantly reduced vs uninfected, age-matched SPF control cats. Four of seven animals with chronic disease that survived >400 DPI developed terminal...
Fig 1. Pathogenesis of experimentally induced feline immunodeficiency disease. (A) Clinical appearance of a cat with advanced AIDS; chronic diarrhea, severe weight loss and debilitation are typical (left); opportunistic infection: necrotizing oral lesions (arrows) involving the tongue (right). (B) Normal lymph node from a control SPF cat; dense cortical and paracortical populations of lymphocytes are apparent (left). Comparable lymph node from a cat with terminal immunodeficiency syndrome; extensive depletion of lymphocytes in the cortical (follicular), paracortical, and medullary areas is evident (right). (C) Prodromal follicular lymphoid hyperplasia in a formalin-fixed, conventionally (H&E)-stained section of spleen from a cat infected with FeLV-FAIDS (left). FeLV-FAIDS replication in splenic follicle from the cat at left demonstrated by immunofluorescence in a methanol-fixed tissue section (right). (D) FeLV-FAIDS-induced lymphoid depletion, severely depleted splenic follicles in hematoxylin and eosin (H&E)-stained histologic sections (left and middle); corresponding immunofluorescence section (right) demonstrates concomitant depletion in viral replication.
Fig 1.  (E) Ablation of the cortical lymphoid follicles (left, arrows) correlated with extinction of viral replication in the same areas (right, arrows), whereas viral replication remained evident in the adjacent nondepleted follicle. (F) Necrotizing enteritis induced by FeLV-FAIDS, with depletion and regeneration of germinal epithelium in the colon (H&E section, left); replication of FeLV in colonic germinal epithelium of (immunofluorescence, right). (G) Replication of feline AIDS virus in salivary gland epithelium (left) and in the epidermis, hair follicles, and sebaceous glands of the nostril of an infected cat (right). Arrowhead – hair shaft (immunofluorescence as above).
lymphoblastic lymphoma superimposed on lymphoid depletion and affecting the marrow, intestine, and mesenteric nodes (see below).

**Virus Tissue Tropism**

Immunofluorescence analysis of lymph node sections from cats inoculated with FeLV-FAIDS revealed intense viral replication (FeLV p27 gag protein) in nodes with many hyperplastic follicles but minimal viral replication in nodes with depleted follicles (Fig 1C through E). The extinction of viral replication in lymphoid tissues, therefore, correlated with extinction of lymphoid follicles. Viral replication in intestinal crypt epithelium also was correlated with evidence of antecedent necrosis of crypt epithelium (Fig 1F).

FeLV-FAIDS replication not associated with discernible cytopathic effect was detected in several cell types: i.e.,...
Fig 3. Sequential changes in blood lymphocyte numbers and blastogenic responses from representative cats inoculated with the mitogens concanavalin A and Staphylococcus protein A in representative FeLV-FAIDS-inoculated SPF cats that developed either persistent viremia (1341) or transient infection and regressive infection (1342) as compared with an uninoculated littermate control.

Table 2. Summary of Hematologic and Immunologic Data in Cats With Immunodeficiency Syndrome Induced by FeLV-FAIDS

<table>
<thead>
<tr>
<th>Parameter Measured</th>
<th>FeLV-FAIDS Viremic Cats</th>
<th>Cats (n)</th>
<th>FeLV-FAIDS Regressors*</th>
<th>Cats (n)</th>
<th>Control SPF Cats</th>
<th>Cats (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lymphocytes/μL (all stages of infection)</td>
<td>3,486 ± 990</td>
<td>20</td>
<td>5,928 ± 213</td>
<td>8</td>
<td>6,579 ± 378</td>
<td>41</td>
</tr>
<tr>
<td>Total lymphocytes/μL (clinical disease)</td>
<td>1,092 ± 494</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total granulocytes + monocytes (all stages of infection)</td>
<td>8,542 ± 483</td>
<td>20</td>
<td>6,520 ± 243</td>
<td>8</td>
<td>11,888 ± 990</td>
<td>41</td>
</tr>
<tr>
<td>Total granulocytes + monocytes (clinical disease)</td>
<td>7,820 ± 519</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%) (all stages of infection)</td>
<td>32 ± 1</td>
<td>20</td>
<td>35 ± 3</td>
<td>8</td>
<td>33 ± 3.7</td>
<td>41</td>
</tr>
<tr>
<td>Hematocrit (%) (clinical disease)</td>
<td>21.3 ± 9.0</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte blastogenesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ConA (all stages of infection)</td>
<td>24,758 ± 3,410</td>
<td>20</td>
<td>33,396 ± 4,447</td>
<td>8</td>
<td>44,822 ± 5,411</td>
<td>41</td>
</tr>
<tr>
<td>ConA (clinical disease)</td>
<td>3,695 ± 1,464</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SpA (all stages of infection)</td>
<td>12,188 ± 3,011</td>
<td>20</td>
<td>29,328 ± 4,321</td>
<td>8</td>
<td>27,473 ± 2,967</td>
<td>41</td>
</tr>
<tr>
<td>SpA (clinical disease)</td>
<td>1,964 ± 1,043</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWM (all stages of infection)</td>
<td>6,933 ± 905</td>
<td>20</td>
<td>11,880 ± 1,464</td>
<td>8</td>
<td>12,990 ± 1,459</td>
<td>41</td>
</tr>
<tr>
<td>PWM (clinical disease)</td>
<td>1,709 ± 827</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total γ-globulin (mg/dL)</td>
<td>0.70 ± 0.9</td>
<td>—</td>
<td>ND</td>
<td></td>
<td>1.58 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Skin allograft rejection (days)</td>
<td>18.5 ± 2.4</td>
<td>—</td>
<td>ND</td>
<td></td>
<td>13.1 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Marrow CFU-e (no. of CFU/10(5) cells seeded)</td>
<td>12 ± 1.7†</td>
<td>—</td>
<td>ND</td>
<td></td>
<td>134 ± 2</td>
<td></td>
</tr>
<tr>
<td>Marrow BFU-e (no. of CFU/10(5) cells seeded)</td>
<td>14 ± 9†</td>
<td>—</td>
<td>ND</td>
<td></td>
<td>82 ± 5</td>
<td></td>
</tr>
<tr>
<td>Marrow CFU-GM (no. of CFU/10(5) cells seeded)</td>
<td>38 ± 8†</td>
<td>—</td>
<td>ND</td>
<td></td>
<td>37 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

*No disease developed after observation periods up to 600 days postinoculation.
†Hematopoietic colony-forming units-erythroid (CFU-e, BFU-e) or granulocyte macrophage (CFU-GM) generated by feline bone marrow cells assayed in a methylcellulose medium culture system. For FAIDS cats, n = 4; for control cats, n = 320.
circulating neutrophils and platelets, salivary gland acinar and duct epithelium, mucosal epithelium of the esophagus, nasal passages, and bronchioles, and the glandular and hair follicle epithelium of the nostrils (Fig 1g). Neither viral antigen nor lesions were found in the testes, ovary, uterus, kidney, liver, adrenal, thyroid, heart, or brain. Thus, the feline AIDS-inducing virus replicated in a range of hemolymphatic and epithelial tissues but appeared to be cytopathic only in bone marrow, intestine, and lymphoid tissue.

The intestinal lesions in cats infected with FeLV-FAIDS resembled those produced by the feline and canine parvoviruses. Therefore, we assayed both original viral inocula and intestinal mucosal extracts from three cats with fatal immunodeficiency syndrome for feline parvoviral antigen by an ELISA sensitive to 2 pg viral antigen. Each was negative. Moreover, six of six SPF cats immunized with commercial feline panleukopenia parvovirus vaccine developed immunodeficiency disease featuring chronic diarrhea and enteritis after FeLV-FAIDS inoculation.

**Southern Blot Analysis of Viral DNA in Tissues**

Southern blot analyses of purified cellular DNA demonstrated viral DNA in all (90 of 90) virus-antigen-positive tissue and bone marrow samples from all 25 viremic cats examined (Table 1). Highest levels of FeLV-FAIDS DNA were in bone marrow, intestine, and lymphoid tissues. As initially reported,⁴⁷ two significant viral genomes were detected in the tissues of infected cats: (a) a ubiquitous “common” or “early” form virus genome recognized as a 3.65-kb internal viral DNA fragment (Fig 4) in all virus-antigen-positive tissues of all viremic asymptomatic and symptomatic cats examined at all postinoculation intervals; and (b) a variant genome (designated variant A) characterized by an additional KpnI site in the extracellular glycoprotein gene (gp70) region of the viral envelope gene and generating a signature 2.1-kb 3′ KpnI fragment (variant A), which was detected in large amounts (15 to 50 copies/cell), predominately as un integrated viral DNA (Fig 4) in the bone marrow, intestine, and nonseverely-depleted lymphoid tissues only of animals with symptomatic immunodeficiency syndrome. In tissues of animals with severe lymphoid depletion, minimal amounts of viral DNA were discernible by Southern blotting and minimal viral antigen was demonstrable by immunofluorescence (Fig 1C through E). Thus, FeLV-FAIDS common-form virus was demonstrated in all viremic cats both before and after the onset of disease, whereas the appearance and amplification of variant A virus genome in marrow, intestine, and lymphoid tissues was correlated strongly with the development of symptomatic, ultimately fatal immunodeficiency disease.

**Late-Onset Neoplasia in Some Animals Infected With FeLV-FAIDS**

Two of the seven animals that developed chronic immunodeficiency syndrome, both of which survived for >400 days after inoculation (471 and 524 days, respectively), also developed terminal T cell or null cell lymphoma affecting the bone marrow, small intestine, and to a lesser degree, mesenteric lymph nodes. Histologic examination of the mesenteric lymph nodes indicated that proliferation of neoplastic lymphoblasts was superimposed on lymphoid involution. Other nodes contained only the lymphoid depletion typical of advanced immunodeficiency syndrome. Southern blot analysis of lymphoma tissues revealed clonal viral integration.
patterns confirming the presence of a clonal proliferation of virus-genome–bearing cells (data not shown). Thus, prolonged FeLV-FAIDS viremia and/or chronic immunosuppression may result in long-latency lymphomagenesis, as has been observed in some HIV-infected patients.66-68

DISCUSSION

Although a lymphoid depletion/immunodeficiency syndrome has been recognized for >15 years in cats infected with feline leukemia virus,1,2,18 the disease has neither been consistently reproduced with FeLV inocula nor has its relationship to virus genotype or other cofactors been elucidated. Cats infected with the most ubiquitous naturally occurring feline leukemia viruses—interference subgroup A (FeLV-A)55-58—commonly remain persistently viremic and asymptomatic for long periods. Viruses of subgroups B or C occur naturally only in combination with FeLV-A57-58 and probably have evolved from the FeLV-A either through mutation or through recombination with silent multicy copy endogenous FeLV sequences in the feline genome (Mullins JI et al, manuscript in preparation, and refs 52 and 58). It is plausible, therefore, that the acute pathogenicity associated with some FeLV field isolates reflects the presence of cytopathic virus variants in these inocula. Further definition of the molecular and biologic properties of FeLV variant genomes should aid in elucidating the mechanisms of disease induction by feline leukemia viruses. The studies we report define the biologic and virologic properties of a naturally occurring intensely immunosuppressive isolate of FeLV which consistently induces fatal immunodeficiency syndrome after incubation periods as short as 3 months to as long as 43 months, an interval governed principally by the age of the cat at time of infection. The onset of clinical immunodeficiency syndrome coincides with the appearance of a characteristic variant viral genome which replicates in bone marrow, lymphoid tissues, and intestine, predominantly as unintegrated viral DNA (UVD). The association of unintegrated retroviral replication with cytopathicity has been demonstrated for reticuloendotheliosis virus,49 visna virus,54 avian osteopetrosis virus,71 and HIV.33 In FeLV-FAIDS–associated immunodeficiency disease, the replication of UVD in target tissues is notable in its magnitude, persistence, and correlation with disease onset and lesions in vivo.

The consistent experimental induction of immunodeficiency disease by a specific type C retrovirus isolate in outbred animals free of intercurrent infection with other pathogens supports the tenet that viral genome rather than host or environmental factors plays the major role in induction of immunodeficiency disease. These results also suggest that neither D type morphology nor a complement of superantigen genes is essential for induction of immunodeficiency syndrome by retroviruses. Our continuing work with FeLV-FAIDS and another feline leukemia virus variant that induces aplastic anemia (FeLV-C-Sarma) suggests that drastic differences in target cell specificity, cytopathic activity, and pathogenicity of FeLVs in vivo are principally the consequence of subtle alterations in the extracellular glycoprotein gene (Riedel NO, manuscripts in preparation).72

Thus, retrovirus glycoprotein gene variants that arise in certain cells, tissues, or stages of infection and possess altered cell tropism and pathogenicity may be responsible for induction of immunodeficiency or other disease syndromes.33,34,70,74,77,78 Both Southern blotting and immunofluorescence results indicate that the principal targets for FeLV-FAIDS variant replication and cytopathic effect (ie, bone marrow, intestine, and lymphoid tissues) are concordant. Apparently, noncytopathic viral replication was detected in several epithelial tissues important to viral excretion and contagious transfer (eg, mucosal epithelium of the pharynx, esophagus, nostrils, salivary gland), as has been demonstrated previously for the Rickard isolate of feline leukemia virus.27 The causal relationship, if any, between unintegrated retroviral DNA replication and host cell death, originally identified by Keshet and Temin49 in cell cultures infected with avian reticuloendotheliosis virus, remains unclear. The presence of UVD per se may not be directly responsible for cytotoxicity, since UVD in the absence of cell degeneration has been observed in HIV-infected T cell lines78 and in the proliferative bone lesions of avian osteopetrosis.71 The unintegrated replication of an immunodeficiency disease–associated FeLV genome in target tissues of infected cats does, however, provide an additional stimulus for study of the relationship between retroviral nonintegrative replication and genesis of cytopathic disease.

The correlation among FeLV-FAIDS replication in intestinal germinal epithelium, persistent diarrhea, and necrosis of mucosal epithelium detected in infected cats suggests that intestinal tropism may be a primary rather than exclusively a secondary manifestation of immunodeficiency disease–inducing retroviruses. The association of AIDS with enteropathy, chronic diarrhea, and intractable weight loss, now recognized as the chief presenting symptom characteristic of African AIDS,11 has been considered secondary to opportunistic infections associated with immunosuppression (eg, cryptosporidiosis). In SPF cats with FeLV-FAIDS–associated enteritis, unlike the situation in naturally infected people or cats with immunodeficiency syndrome, neither pathogenic bacteria, viruses, nor parasites are associated with or required for development of the intestinal lesions. Although the necrosis and regeneration of the intestinal crypt epithelium in FeLV-FAIDS–infected cats resembles that caused by feline parvovirus (an indigenous feline virus which also infects mitotically active cells and could potentially be responsible for enteritis in cats naturally infected with FeLV), our studies indicate that the feline parvovirus is not involved in FeLV-FAIDS–associated enteropathy. In that intestinal crypt cell degeneration also has been associated with the enteropathy affecting many AIDS patients,64 further consideration should be given to intestinal epithelium as a target tissue for cytopathic retroviruses.

FeLV-associated immunodeficiency syndrome, therefore, can be rapidly and consistently transmitted experimentally with a naturally occurring isolate of feline leukemia virus. The course of feline AIDS varies with and can be manipulated by the age of the animal at time of infection. Our results further suggest that the onset of clinical disease is
associated with the replication, first in bone marrow and then in lymphoid tissues and intestine, of a disease-specific viral variant, the accelerated cytopathic replication of which is responsible for precipitation of immunodeficiency disease. It seems probable that such immunosuppressive FeLV variants are generated continually in nature and that previous experimental studies in which AIDS-like syndromes have been induced in cats may have involved inocula containing similar lymphocytotoxic virus genomes. It also seems plausible that similar mechanisms involving more and less pathogenic, and possibly defective, variants of the human AIDS retroviruses are involved in onset and progression of human AIDS.

Since this article was submitted, Pedersen et al. have reported the isolation of a feline lentivirus-like virus (designated FTLV) from a group of cats with multiple chronic infections suggestive of underlying immunosuppression syndrome. This newly recognized non-FeLV feline retrovirus, which contains a Mg\(^{2+}\)-dependent reverse transcriptase, is of type D morphology, has been propagated only in freshly isolated, mitogen-pulsed feline lymphocytes, and is distinct from the pathogenic FeLV isolate we describe. FeLV-FAIDS transcriptase strongly prefers Mn\(^{2+}\) catalysis, is of C type morphology, replicates efficiently in fibroblasts as well as lymphocytes, contains characteristic FeLV gag and env proteins, and hybridizes with a DNA probe specific for exogenous FeLV LTR U3-sequences.\(^{47}\) Cats inoculated with the original FeLV-FAIDS inoculum, subpassaged virus, and normal SPF cats are negative for FTLV antibody by immuno-fluorescence and Western blotting assays (performed in the laboratory of Dr Niels C. Pedersen). Neither the original FeLV-FAIDS inoculum nor inoculated lymphocyte or fibroblast cultures generate Mg\(^{2+}\)-dependent reverse transcriptase activity. Moreover, the full-length genomes of the FeLV-FAIDS common form and variant A viruses described here have recently been molecularly cloned (Overbaugh JM et al, manuscript in preparation) and the variant virus shown to be capable of producing both lymphocyte killing in vitro and fatal immunodeficiency syndrome in vivo. Characterization of the pathogenic determinants of this immunodeficiency-inducing feline leukemia virus should contribute significantly to the understanding of retrovirus-mediated immunodeficiency diseases.

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