Replication kinetics and cell tropism of an immunosuppressive feline leukaemia virus

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To elucidate in vivo cell tropism and infection kinetics of an immunodeficiency-inducing isolate of feline leukaemia virus (FeLV-FAIDS), we quantified the two major genotypes comprising FeLV-FAIDS [the replication-competent common form (clone 61E) and the replication-defective variant (clone 61C)] in lymphocyte and leukocyte populations from infected cats. Micromagnetic separation of cell subsets, virus genome-specific PCR and flow cytometry were used to demonstrate the following sequence of events in infected animals: (i) very early replication of both 61E and 61C in CD4 T cells (provirus burden 0–2 to 1 copy/cell at 2–4 weeks post-infection); (ii) lower magnitude replication of both viruses in CD8 T cells and B cells during this initial phase of infection; (iii) plateauing of CD4 cell virus burden accompanied by escalation in CD8 and B cell provirus burdens after 4 weeks; (iv) extensive infection of haemopoietic and circulating myeloid cells. FeLV-FAIDS 61E and 61C replication kinetics and lymphocyte tropisms were similar in blood and lymph nodes, where provirus burdens ranged from 0·15 to 1·0 copy/cell. Moreover, virus infection was productive; 8–48% of blood lymphocytes, 35–81% of node lymphocytes and 53–98% of bone marrow cells expressed FeLV capsid antigen (p27 Gag). These findings suggest that the immunosuppressive potency of FeLV-FAIDS reflects the unique cytopathicity rather than unique cytotropism of its 61C (versus 61E) component.

Introduction

While the pathogenic mechanisms responsible for the development of feline leukaemia virus (FeLV)-induced immunodeficiency remain incompletely understood, early virus targeting to lymphoid and haemopoietic cells must be a prerequisite to immunosuppression.

We have recently demonstrated that an immunosuppressive strain of FeLV (FeLV-FAIDS) infects several subclasses of circulating lymphocytes, including CD4+ and CD8+ T cells and IgG+ (probably B) cells (S. Quackenbush, J. Mullins & E. Hoover, unpublished results). The FeLV-FAIDS isolate, however, is comprised of two major virus genotypes: a highly replication-competent subtype A virus (prototype clone 61E) and a family of replication-defective major variant viruses closely related to 61E (molecular clone 61C is the prototype). Clone 61E is not acutely pathogenic but induces long latency lymphoma (Hoover et al., 1987; Jarrett et al., 1964; Mullins et al., 1991; Overbaugh et al., 1988). Construction of replication competent chimeras containing the 61C env gene have demonstrated that 61C is T cell-cytopathic in vitro, induces acute immunosuppression in vivo, and that its major pathogenic determinants localize within the surface glycoprotein (SU, gp70; Donahue et al., 1991; Hoover et al., 1987; Mullins et al., 1986, 1991; Overbaugh et al., 1988; Quackenbush et al., 1990). In vivo infection studies have correlated the development of clinical disease with the appearance of unintegrated virus DNA (UVD) in bone marrow, lymphoid tissues and intestine (Hoover et al., 1987; Mullins et al., 1986, 1991; Overbaugh et al., 1988; Quackenbush et al., 1989). Previous studies of FeLV-FAIDS-induced immunodeficiency have demonstrated an early decrease in CD4+ lymphocytes and an early deficit in primary antibody response generation to T cell-dependent antigens (Pardi et al., 1991; Quackenbush et al., 1990). Subsequent studies demonstrated that this functional impairment preceded the decrease in
circulating CD4+ T cells and involved impaired production of B cell stimulatory cytokines normally produced by the T helper 2 cell population (Diehl & Hoover, 1992).

Therefore, to elucidate further the pathogenesis of FeLV-FAIDS infection in vivo, we used cell sorting, semi-quantitative PCR and two-colour flow cytometry to analyse longitudinally the kinetics and targeting of FeLV-FAIDS 61E and 61C to lymphoid and haemopoietic cell subsets.

Methods

Animals, virus inoculation and sampling. The replication-competent chimeric virus comprised of the left half (5'LTR–gag–pol) of 61E and the right half (env–3'LTR) of 61C produces a rapidly fatal leukenopia and enteritis in infected cats (Overbaugh et al., 1988). Therefore, we chose not to use this virus to assess the distribution of variant 61C. Rather, a mixture of clones 61E and 61C generated by transfection with 61C followed by superinfection with 61E was used (designated 61E/C; Overbaugh et al., 1988) to give longer survival and thus an infection course more similar to that of 61E. Twenty 10-week-old SPF cats obtained from Liberty Laboratories were used in this study. The study groups were as follows: (i) uninfected controls (n = 6); (ii) FeLV-FAIDS 61E-infected cats (n = 7); and (iii) FeLV-FAIDS 61E/C-infected cats (n = 7). Cats were anaesthetized with ketamine hydrochloride (25 mg/kg) and injected intravenously with 106 infectious units (Fischinger et al., 1974) of either FeLV-FAIDS 61E or 61E/C (Overbaugh et al., 1988). Complete blood counts, differential leucocyte counts and lymphocyte immunophenotyping were performed monthly. The animals were euthanized between 18 and 27 weeks post-inoculation (p.i.) by intravenous barbiturate overdose and necropsy.

Monoclonal antibodies (MAbs). The MAbs used in these studies were as follows: (i) anti-CD4 (3.4F4.5; O'Reilly & Hoover, 1993); (ii) anti-CD8 (31B.5; O'Reilly & Hoover, 1993); (iii) anti-CD21 (CA2.1Db) generated against canine cells, which react with canine and feline B cells (kindly provided by P. F. Moore, University of California-Davis, Davis, Calif., USA); (iv) FeMy (A73B1), which recognizes feline myeloid cells (monocytes and granulocytes) (Groshek et al., 1995); (v) anti-CD20 (10-1D2; O'Reilly & Hoover, 1993); (vi) anti-CD11b (3-1F10; O'Reilly & Hoover, 1993); (vii) FeLy (E10A3), which recognizes feline bone marrow lymphocytes (Groshek et al., 1995); and (viii) FeEr (G4C1), which recognizes feline bone marrow erythroid cells (Groshek et al., 1995).

Cell isolation and purification. Peripheral blood mononuclear cells (PBMCs) were isolated from blood collected in EDTA by density gradient centrifugation as previously described (Quackenbush et al., 1989). PBMC were washed four times with PBS, resuspended in lymphocyte medium composed of RPMI 1640 supplemented with 10% fetal calf serum (Hyclone Laboratories), 2% glutamine (200 mm) and 1% penicillin–streptomycin (1 U/ml) and enumerated. Bone marrow was collected from the femur or humerus by aspiration biopsy into a syringe containing heparin. The bone marrow was diluted with 20 ml PBS and layered over 10 ml Histopaque 1177 (Sigma) and cells were isolated as above. Mesenteric lymph node was collected at necropsy in cold RPMI 1640 medium supplemented with 2% penicillin–streptomycin, minced into small pieces and passed through wire mesh (Collector, E-C Apparatus) to obtain single cell suspensions. The cells were washed four times with PBS, resuspended in the above medium and enumerated.

Cell subsets were purified using magnetic separation columns (MiniMACs; Miltenyi Biotechnology). From 1–5 x 106 PBMC, lymph node or bone marrow cells were added to microfuge tubes and washed with 1 ml PBS containing 2% fetal calf serum and 0.02% NaN3 (PBS/FCS/NaN3). The cells were resuspended in 100 l of PBS/FCS/NaN3, supplemented with 30–50 l (30 l per 106 cells plus 5 l each additional 106 cells) of MAb conjugated to either fluorescein isothiocyanate (FITC) or phycoerythrin (PE), and incubated for 30 min at 4 °C. Cells were washed twice with 1 ml PBS/FCS/NaN3 and resuspended in 100 l PBS/FCS/NaN3. Ten l of rat anti-mouse IgG1 or rat anti-mouse IgG MACS magnetic microbeads (Miltenyi Biotechnology) were added to each tube and incubated for 20 min at 4 °C. Cells were washed twice with 1 ml PBS/FCS/NaN3 and resuspended in 500 l of PBS with 0.5% BSA (PBS/BSA). Each column was then washed with 500 l of PBS/BSA, the cell suspension added and the column washed again with 500 l PBS/BSA. The eluate was collected as the non-magnetic fraction. The column was then washed six times with 500 l of PBS/BSA and the eluate discarded. After removing the column from the magnetic separator, 1 ml PBS/BSA was added and the detached, formerly adherent, cells were collected in a microfuge tube with the aid of a plunger. Cells were enumerated using a Coulter ZM particle counter. The purity of the separation was assessed with a Coulter Epics Profile II flow cytometer. Each cell population used for PCR analysis was purified to ≥ 98%.

Cell lysate PCR, amplification, hybridization and detection. Purified cells (10 or 20 x 106) were added to microfuge tubes and pelleted. The pellets were then resuspended in 50 or 100 l of PCR lysis buffer (45 mm-NaCl, 9 mm-Tris–HCl pH 8.3, 2.2 mm-MgCl2, 0.1 mg/ml gelatin, 0.4% Nonidet P-40, 0.4% Tween 20, 1 mg/ml proteinase K). Tubes were placed in a 60 °C water bath for 12–15 h, treated at 95 °C for 10 min and frozen at −20 °C (Zack et al., 1992). The PCR mixture consisted of 50 mm-KCl, 10 mm-Tris–HCl pH 8.3, 1.5 mm-MgCl2, 0.01% gelatin, 200 mm of each dNTP, 10 pmol of each primer and 2–3 units AmpliTaq polymerase (Perkin Elmer). Twenty-five l of cell lysate was added to each PCR reaction in a total volume of 100 l, which was then overlaid with mineral oil. Each amplification consisted of 30 s at 94 °C, 20 s at either 57 °C (TR1/Q1 primers) or 59 °C (TR3/Q1 primers), 20 s at 72 °C for 30 cycles and 5 min at 72 °C for 1 cycle (Reinhardt et al., 1993). The oligonucleotides used for PCR amplification were as follows: (i) 61E virus-specific primer TR1 (nucleotide positions 6260–6277) 5' AATGATTAACCGGGCCA 3'; (ii) 61C virus-specific primer TR3 (nucleotide position based on the 61E sequence 6252–6259 plus 6278–6287) 5' GCCAACCCGGTACCCTC 3'; and (iii) Q1 primer (nucleotide position 6415–6397 based on the 61E sequence) 5' CCCATCGTTGCCCCCTCA 3'. A 155 bp region of the FeLV 61E env gene or a 145 bp region of the FeLV 61C env gene was amplified using these virus-specific oligonucleotides (S. Quackenbush, J. Mullins & E. Hoover, unpublished results).

Amplified products were hybridized in solution with the 32P-labelled oligonucleotide probe Q32 (GACAGACGGGTGCTTCCCCCTTA CACCCTCGCCT), which hybridizes to a conserved region of the env gene of both 61E and 61C and is internal to the primers used for amplification. The Q32 probe was end-labelled using a 5' end-labelling kit (Boehringer Mannheim) according to manufacturer's instructions. PCR-amplified products were phenol–chloroform extracted twice. Twenty l of amplified product and 10 l of hybridization mixture (4 l 60 mm-NaCl, 0.8 l 40 mm-EDTA, 2.5 x 106 c.p.m. Q32) were incubated at 95 °C for 5 min and placed in a 56 °C water bath for 30 min. Samples were subjected to electrophoresis in 10% acrylamide gels at 150 V for 4 h, fixed in 10% acetic acid and exposed to film overnight. All experimental samples were done in triplicate. Films were scanned and analysed using the Scan Analysis 6800 software (Biosoft).
For quantification of the 61E and 61C provirus loads in purified cell populations, clones of the feline T cell line 3201, containing either the 61E or 61C virus genomes, were used (S. Quackenbush, J. Mullins & E. Hoover, unpublished results). A standard curve for each PCR reaction was generated from densitometry readings of twofold dilutions of 61E-B10 and 61C-D9 lysates.

Two-colour flow cytometric analysis. 5–10 × 10^6 PBMC lymph node or bone marrow cells were added to microfuge tubes and washed with 1 ml PBS/FCS/NaN₃. Cells were resuspended in 30 μl of FITC-conjugated MAbs and incubated at 4 °C for 30 min. Cells were washed twice with 1 ml PBS/FCS/NaN₃ and fixed in 500 μl of 2% paraformaldehyde in PBS at 4 °C for 1 h. Cells were then washed as above and resuspended in 30 μl of PE-conjugated MAb (24-A2) to FeLV CA (p27 Gag) (a gift from N. C. Pedersen, University of California-Davis; Lutz et al., 1983). The cells were incubated at 4 °C for 30 min, washed twice with 1 ml PBS/FCS/NaN₃, resuspended in PBS and analysed by flow cytometry.

Isolation of macrophages. Six-well plates (Falcon) were coated with 10 μg/ml of cat IgG (Jackson Laboratories) in PBS for 30 min at room temperature and rinsed with 2 ml PBS. PBMC were isolated as described above, plated at 4–8 × 10^6 cells/well and incubated at 37 °C for 1 h. Plates were washed three times with 2 ml PBS and 2 ml/well medium (described above) was added. Cultures were placed at 37 °C for an additional 48 h. Medium was removed and plates were washed twice with 2 ml PBS. To detach macrophages a 2% lidocaine solution, diluted 1:5 with PBS and 2 ml/well, was added and incubated at 37 °C for 30 min. Detached macrophages were pelleted, washed in PBS, enumerated and prepared for PCR as described above.

Statistical analysis. Statistical analyses were performed by using Student’s t-test. A P value of ≤ 0.05 was considered significant.

Results

FeLV-FAIDS disease induction

All seven of the FeLV-FAIDS 61E-inoculated cats and six of seven FeLV-FAIDS 61E/C-inoculated cats became persistently viraemic. None of the 61E-infected animals developed clinical signs of disease during the 6 month course of study. Four of the six 61E/C-infected cats developed FeLV-related disease during this period. One animal (APOP) developed multicentric lymphoma at 18 weeks p.i. Immunophenotyping identified the tumour cells as B cells [positive for the B cell markers CD20 (MAb 10-1D2) and CD21 (MAb CA2.1D6) and negative for CD4 and CD8].

Southern blot analysis

Southern blot analysis was performed on bone marrow DNA from all cats at study termination. Clone 61E was present in all viraemic animals. Clone 61C was detected as UVD in all four 61E/C-infected cats that developed clinical disease, a previously demonstrated hallmark of FeLV-FAIDS immunodeficiency (Hoover et al., 1987; Mullins et al., 1986, 1991; Overbaugh et al., 1988; Quackenbush et al., 1989).

Effects of infection on blood and tissue lymphocyte subset numbers

Total lymphocytes. Total lymphocytes of the uninfected control cats underwent the normal age-related increase through week 6, then stabilized throughout the remainder of the study (Fig. 1a). Total lymphocytes of both the 61E- and 61E/C-infected cats decreased between weeks 2 to 4 through weeks 12 to 15 (Fig. 1a).

Lymphocyte subsets. In the 61E/C-infected cats, CD4 and B cells decreased to 25% below controls between weeks 4 and 12 then rose to within the control range thereafter (Fig. 1b, d). CD8 lymphocytes gradually rose to levels greater than those of control cats by 12 weeks (Fig. 1c). Lymphocyte subset changes in 61E-infected cats were less pronounced, usually remaining within the range of the control group (Fig. 1).

Red blood cells. Four of the six viraemic 61E/C cats had significantly lower hematocrit values (range 11–27) than either control (range 30–34) or 61E-infected cats (range 26–34) throughout the study. The hematocrit values of 61E-infected cats were not significantly different from the control animals, with the exception of one cat that maintained a subnormal hematocrit level throughout the study.

Provirus burdens in lymphoid and bone marrow cell populations

To determine the kinetics of FeLV-FAIDS 61E and 61C virus burden early after infection, PCR analysis was performed in triplicate on blood lymphocyte subpopulations from sequential blood samples collected from each animal. Lymphocyte subsets were purified using MAbs and micromagnetic bead separation columns. Provirus was detected using 61E- and 61C-specific primers in semi-quantitative PCR.

Blood lymphocytes: 61C provirus. The 61C virus DNA was first detected at week 4 in two of six 61E/C-infected cats and in all 61E/C cats thereafter. The CD4 T cell population contained the highest initial 61C provirus burden [mean 16600 ± 22600 copies (cc)10^6 cells] in these two cats (Fig. 2a). Initial replication of 61C to higher levels in CD4 cells was a consistent finding in the other four 61E/C-infected cats, although at different times p.i. The 61C provirus load increased gradually in CD8 cells throughout the course of infection. Low levels of 61C provirus were present in B cells until 15 weeks p.i., but then reached levels (mean 21900 ± 29400 cc/10^5 cells) comparable to that in T cells (mean 33600 ± 30180 cc/10^5 cells; Fig. 2a). By 24 weeks p.i., all lymphocyte subpopulations contained similar 61C burdens (CD4 mean 55500 ± 34300; CD8 mean 54500 ± 35900; B cell mean 47100 ± 2000 cc/10^5 cells; Fig. 2a).

Blood lymphocytes: 61E provirus. No significant differences in the provirus burden or replication kinetics of 61E were detected between the 61E- and the 61E/C-infected cats. Therefore, the
61E data from both groups was combined. As with 61C, the highest initial (2 weeks p.i.) 61E provirus burden was detected in CD4 cells in all cats infected with either 61E or 61E/C (mean 99,000 ± 93,000 provirus cc/10^5 cells; Fig. 2b). This initial provirus burden in CD4 cells was significantly higher than in CD8 or B cells. In CD8 cells, the mean provirus burden was approximately sixfold lower than in CD4 cells (mean 17,000 ± 12,600 cc/10^5 cells; Fig. 2b). The B cell population carried the lowest (yet still substantial) mean provirus load of 9500 ± 6200 cc/10^5 cells (Fig. 2b). At 24 weeks, the mean 61E provirus loads were very high in T cells (CD4 mean 75,000 ± 35,000; CD8 mean 77,100 ± 36,400 cc/10^5) and B cells (114,300 ± 51,900 cc/10^5) in 61E- and 61E/C-infected cats (Fig. 2b).

**Lymph node: 4 weeks p.i.** Lymphadenopathy was detected in all cats early in infection. Lymph nodes were excised at 4 weeks from one 61E- and one 61E/C-infected cat to determine virus burden. Both 61E and 61C proviruses were detected at the highest levels in CD4 cells (61E mean 122,100 ± 66,610; 61C mean 110,100 cc/10^5 cells) followed by CD8 cells (61E mean 71,100 ± 28,850; 61C mean 65,700 cc/10^5 cells) and B cells (61E mean 28,250 ± 10,390; 61C 5500 cc/10^5 cells).

**Lymph node: terminal 61C provirus.** The 61C provirus was detected in CD4, CD8 and B cells from all cats at the termination of the study (CD4 mean 34,700 ± 33,500; CD8 mean 20,100 ± 14,000; B cell mean 26,100 ± 35,900 cc/10^5 cells; Fig. 3).

**Lymph node: terminal 61E provirus.** Again, the 61E provirus burden was similar in all lymph node cell subsets examined from 61E- and 61E/C-infected cats. The 61E provirus burden in T cells was similar (CD4 mean 51,300 ± 43,000; CD8 mean 45,400 ± 68,400 cc/10^5 cells), whereas B cells had a significantly higher virus load (mean 102,600 ± 84,600 cc/10^5 cells; Fig. 3).
Bone marrow: terminal 61C provirus. The 61C provirus was detected in bone marrow lymphoid, erythroid and myeloid cells. In two of the three 61E/C cats, provirus burden in erythroid and myeloid cells was extremely high (mean 226550 ± 94400 and 226050 ± 72760 cc/10⁵ cells, respectively; Fig. 4). The two cats with high provirus burden had hematocrit values of 11 and 28, as well as the presence of UVD in bone marrow. The third cat had a normal hematocrit and very little 61C provirus in lymphoid, erythroid or myeloid cells.

Bone marrow: terminal 61E provirus. 61E provirus also was detected in bone marrow lymphoid, erythroid and myeloid cell populations. In general, the marrow erythroid and myeloid populations contained more provirus DNA (erythroid mean 99060 ± 50190; myeloid mean 92840 ± 46260 cc/10⁵ cells) than did the lymphoid population (mean 57440 ± 55780 cc/10⁵ cells; Fig. 4).

Macrophages: 61E and 61C proviruses. Macrophages were isolated from blood of cats at 25–27 weeks p.i. by adherence to plastic culture dishes coated with anti-feline Ig. The 61E and
61C provirus burdens were moderate (61E $28,300 \pm 2,200$; 61C $15,000 \pm 19,000$ cc/$10^6$ cells).

**Proivirus in lymphoma cells.** Both the 61E (9 cc/cell) and 61C (1 cc/cell) virus genomes were identified in the B cell lymphoma developed by animal APO1. The tumour cells also expressed FeLV SU (gp70) as identified by MAb C11D8 (Grant et al., 1983).

In summary, analysis of blood and lymph node cell fractions revealed both the 61E and 61C proviruses present in all populations. Initially, both viruses replicated to higher levels in CD4 cells. This was followed by a gradually escalating provirus burden in CD8 cells and finally in B cells. Ultimately, the virus load in B cells became greater than (61E) or equal to (61C) that in T cells and both 61C and 61E loads were extremely high in bone marrow erythroid and myeloid progenitor cells.

**FeLV antigen expression in lymphocytes and other cell populations**

**Blood.** The percentage of CD4, CD8, B cells and monocytes that expressed FeLV CA (p27) was determined at 4, 8, 12, 15 and 24 weeks p.i. (Fig. 5). Although there was considerable variation in the percentage of FeLV CA-positive CD4, CD8 and B cells among cats, the mean percentage virus antigen-positive cells was higher in the 61E/C-infected than in the 61E-infected cats (Fig. 5). The number of T cells that expressed FeLV CA increased gradually throughout infection in both 61E- and 61E/C-infected cats, such that by 24 weeks 26–36% of CD8 and CD4 cells were positive. Surprisingly, yet in parallel with provirus data, more B cells from both 61E- and 61E/C-infected animals expressed virus antigen than did T cells. The percentage of circulating CD11b+ monocytes that
exhibited FeLV p27 progressively reached very high levels in both 61E- and 61E/C-infected animals; 92 ± 5% were positive for p27 by 24 weeks p.i. (Fig. 5). Virtually all granulocytes (98 ± 1%) from all infected cats were also positive for p27.

**Lymph node.** A large fraction of mesenteric lymph node cells contained FeLV CA (Fig. 6), thus paralleling the findings in circulating lymphocytes (Fig. 5). In 61E/C-infected cats, 54%, 47% and 81% of CD4, CD8 and B lymph node cells, respectively, expressed p27. This compared with 40%, 35% and 66% of CD4, CD8 and B cells in 61E-infected cats, respectively.

**Bone marrow.** In bone marrow, FeLV CA was significantly more frequent in erythroid cells from 61E/C-infected cats (mean 85%) than from 61E cats (mean 53%). A mean of 96% and 97% bone marrow CD11b+ myeloid cells were also FeLV p27-positive in 61E- and 61E/C-infected cats, respectively.

In summary, FeLV protein was produced in CD4, CD8 and B cells from blood and lymph node as well as in circulating monocytes, granulocytes and bone marrow erythroid and myeloid cells. The number of FeLV CA-positive cells increased with time. Overall, a higher percentage of cells from 61E/C-infected cats expressed CA than did cells from 61E-infected cats. Provirus and Gag expression data correlated.

### Discussion

The kinetics of FeLV-FAIDS replication in circulating lymphocytes can be summarized as follows: (i) virus replicated in CD4 cells early (by 2 weeks) after infection, with the provirus burden approaching 1 cc/cell; (ii) after the initial burst of virus replication in CD4 cells the provirus burden decreased slightly and reached a plateau thereafter; (iii) virus replication also occurred early in CD8 and B cells but the magnitude was lower and kinetics slower than in CD4 cells; (iv) ultimately, however, the provirus load in B cells exceeded or equalled that in T cells (≥ 1 cc/cell).

The broad tropism of FeLV-FAIDS for T cells, B cells, monocytes, macrophages, bone marrow lymphoid, erythroid cells and myeloid cells is likely to be neither unique to FeLV nor to this particular FeLV isolate. The simian type D retrovirus (SRV-1) appears to have a similar lymphocyte tropism in infected rhesus macaques; virus was isolated from CD4, CD8 and B cell populations (Maul et al., 1988). SRV-1 appears to differ from FeLV in that neither granulocyte nor platelet infection has been demonstrated (Hardy, 1993; Hoover et al., 1987; Maul et al., 1988; Rojko et al., 1978). The ts1 mutant of Moloney murine leukaemia virus (MuLV), which induces immunodeficiency and hindlimb paralysis, also exhibits early tropism for CD4+ T cells (Saha & Wong, 1992), whereas the immunosuppressive LP-BM5 MuLV extensively infects B cells (Cheung et al., 1991; Huang et al., 1991) as well as CD4+ T cells and macrophages (Kim et al., 1994; Kubo et al., 1992). Two human oncoretroviruses are generally considered to be T lymphotropic. Richardson et al. (1990) detected human T lymphotropic virus type I (HTLV-I) provirus by PCR in CD4 T cells but not in CD8 T cells, B cells or monocytes. In contrast, CD8+ lymphocytes appear to be the preferential target in HTLV-II-infected patients (Ijichi et al., 1992). However, recently the in vivo cellular tropism of these two viruses has been extended to include CD4+, CD8+ and B cells (Casoli et al., 1995; Koyanagi et al., 1993). Casoli et al. (1995) also suggest that the expansion of HTLV-II infection to CD19+ B cells may correlate with an increase in provirus load. The feline immunodeficiency lentivirus (FIV) appears to exhibit both T and B cell tropism but the FIV virus burden is 100- to 10000-fold lower than that for FeLV (Dean et al., 1993; English et al., 1993). Interestingly, FIV tropism studies also suggest that CD4 cells harbour a higher provirus load early in infection and that B cells may become a major reservoir in chronically infected cats (Dean et al., 1993; English et al., 1993). While B cells can be infected by human immunodeficiency virus (HIV; Poulin et al., 1994), this is thought to be a very minor host cell population overall. Thus, among a broad array of retroviruses, CD4+ T lymphocytes appear to be a frequent early target cell whereas B cells are subsequent reservoirs for some retroviruses.

The progressive increase in the number of T lymphocytes expressing FeLV Gag in 61E/C-infected cats (versus 61E-infected) may reflect progression of immunodeficiency. In HIV infection, gag mRNA is detectable in more patients with AIDS than in asymptomatic patients (Schnittman et al., 1991) and the production of more HIV genomic RNA correlates with disease progression (Michael et al., 1992). Likewise, in simian immunodeficiency virus (SIV)-infected macaques, more rapid disease progression correlates with increased numbers of productively infected cells in lymph nodes (Chakrabarti et al., 1994; Hirsch et al., 1995). Provirus burden in lymph nodes of FeLV-FAIDS 61E/C-infected cats was 0.2 to > 1 cc/cell and 35% to 80% of lymph node cells expressed virus antigen. FeLV Gag expression in B cells and CD4+ T cells was approximately equivalent in lymph node and PBMC. Thus in FeLV-FAIDS infection, perhaps in contrast to lentiviruses (Chakrabarti et al., 1994; Embretson et al., 1993; Pantaleo et al., 1991, 1993), virus burdens in peripheral blood and lymphoid tissues appear to be similar.

We used concurrent cell-surface phenotype and intracellular FeLV Gag labelling to demonstrate FeLV replication in circulating monocytes and neutrophils and in marrow erythroid and myeloid cells by flow cytometry. These results confirm and extend those of Rojko et al. (1978, 1979) who localized FeLV Gag in tissue sections by immunofluorescence. Also in agreement is the work of Dean et al. (1992) who used light scatter characteristics to identify the major cell lineages with concurrent intracellular p27 labelling to identify FeLV-replicating cells by flow cytometry. The presence of p27 as well as the detection of 61E and 61C genomes in macrophages
Fig. 6. Expression of FeLV CA in lymphocyte subsets from mesenteric lymph node. 61E-infected cats (n = 7) are represented by the open symbols and 61E/C-infected cats (n = 5) by the closed symbols. The black bar represents the mean of each group. Data is presented as the percentage of the lymphocyte subset that expresses FeLV CA.

and neutrophils (data not shown) confirms these cells are productively infected. We found that in bone marrow of 61E/C-infected cats, the highest 61C provirus burden (1·6 to 2·9 cc/cell) was in erythroid and myeloid cells. Southern blot analysis of marrow DNA demonstrated 61C at high copy number and primarily as UVD, a characteristic of FeLV-FAIDS infection. While bone marrow lymphocytes also contained the 61C (as well as 61E) provirus, the level was lower than that in erythroid or myeloid cells. Thus, the bone marrow cells harboring the FeLV-FAIDS variant virus at high copy UVD appear to be primarily myelopoietic rather than lymphoid cells. These findings do not support a direct relationship between UVD production in marrow lymphoid progenitor cells and systemic lymphoid depletion.

FeLV shares tropism for haemopoietic cells with other oncoretroviruses. Spleen focus-forming virus, a recombinant between Friend MuLV and endogenous MuLV env-like sequences, replicates in erythroid progenitors and induces erythroleukaemia (Wolff & Ruscetti, 1988). Avian erythroleukaemia virus infects erythroid progenitor cells where it activates the c-erbB oncogene by LTR insertion (Fung et al., 1983), whereas avian myeloblastosis virus transforms myeloid progenitors (Payne, 1992). SIVmac infects marrow myeloid cells of macaques, in which suppressed haemopoietic progenitor cell colony formation in vitro has been demonstrated (Watanabe et al., 1990). Subgroup C FeLV infects and depletes or suppresses erythropoietic cells, resulting in fatal red cell aplasia (Abkowitz et al., 1987; Dean et al., 1992; Hoover et al., 1974; Jarrett et al., 1984; Onions et al., 1982; Reidel et al., 1986). Thus, oncoretrovirus infection of bone marrow progenitor cells appears to be common and may lead to several pathogenic consequences.

The present study establishes that very early after infection, both the common and variant forms of FeLV-FAIDS replicate in large fractions of CD4+ T cells, but also target CD8+ T cells, B cells, macrophages and haemopoietic cells. This broad tropism and high level of virus replication is consistent with the lymphoid and haemopoietic dysfunction and depletion which characterizes FeLV-FAIDS and other FeLV infections. While in vitro infection studies suggest that failure to establish superinfection interference may be involved in the T cell cytopathicity of 61C (Reinhart et al., 1993; Kristal et al., 1993), the molecular mechanisms that operate in vivo require further investigation.

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