Retrovirus-Induced Feline Pure Red Blood Cell Aplasia: Pathogenesis and Response to Suramin

By Janis L. Abkowitz

Feline leukemia virus, subgroup C/Sarma (FeLV-C/Sarma) induces pure red blood cell aplasia in cats. Although erythroid (BFU-E and CFU-E) and granulocyte/macrophage (CFU-GM) progenitors are infected with this virus, only erythropoiesis is impaired. Two to 3 weeks before the onset of anemia, CFU-E become undetectable in marrow cultures while earlier erythroid progenitors (BFU-E) persist, suggesting that FeLV-C/Sarma (presumably via its envelope glycoprotein gp70) inhibits the differentiation of BFU-E to CFU-E in vivo. To correlate in vitro observations with the progression of disease, prospective studies were performed in six cats. These studies showed that at the time that the frequencies of CFU-E decreased in marrow cultures, BFU-E no longer responded to hematopoietic growth factor(s), although the responses of CFU-GM were unchanged. In further studies, anemic cats received suramin, a reverse-transcriptase inhibitor with other diverse effects. Within 4 to 14 days, erythropoiesis improved and up to 1,616 CFU-E were detected per 10^6 marrow mononuclear cells. However, progenitor cells remained infected, suggesting that suramin modulated erythroid differentiation without inhibiting progenitor infection. These observations led to the hypothesis that the gp70 of FeLV-C/Sarma impairs BFU-E differentiation by interference with ligand/receptor interactions or signal transduction pathways unique to erythroid cells. Understanding this mechanism should provide insights into the interactions controlling early erythropoiesis.

FEELINE LEUKEMIA virus (FeLV) is a retrovirus that is horizontally transmitted among cats. Cats viremic with FeLV develop a wide spectrum of hematologic diseases, including leukemia, lymphoma, myelodysplastic syndromes, immunosuppression, and nonregenerative anemia. Subgroups of FeLV (designated A, B, and C) have been defined by viral interference patterns and by the species-specificity of host cells. Viruses from various subgroups differ primarily in the structure of their envelope glycopro
cipits, including FeLV-C/Sarma (presumably via its envelope glycoprotein gp70) and FeLV-C/Sarma (presumably via its envelope glycoprotein gp70) inhibit the differentiation of BFU-E to CFU-E in vivo. To correlate in vitro observations with the progression of disease, prospective studies were performed in six cats. These studies showed that at the time that the frequencies of CFU-E decreased in marrow cultures, BFU-E no longer responded to hematopoietic growth factor(s), although the

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From the Division of Hematology, Department of Medicine, University of Washington, Seattle. Submitted June 15, 1990; accepted November 29, 1990. Supported by Grant HL31823 from the Heart, Lung, and Blood Institute of the National Institutes of Health.

Part of this study has been published previously (Blood 72:326a, 1988 [abstr, suppl 1]). Address reprint requests to J.L. Abkowitz, MD, Division of Hematology, Mail Stop RM-10, Department of Medicine, University of Washington, Seattle, WA 98195.

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0006-4971/91/7707-0001$3.00/0

cats and one G-6-PD heterozygous cat. Once anemia developed, the cats were treated with suramin. Suramin is a reverse-transcriptase inhibitor and, hence, should block replication to viral DNA and integration into the host genome. The initial purpose of these studies was to determine the level of differentiation at which cells were infected with FeLV-C/Sarma. We reasoned that if self-renewing hematopoietic stem cells were infected and had integrated viral DNA into their genome, progeny of these cells, including BFU-E and CFU-GM, would inherit proviral DNA, and suramin should have no effect. Alternately, if cells were infected near the BFU-E and CFU-GM stages of differentiation, a time when more rapid cell division favors the integration of retroviral DNA, suramin could prevent infection and reverse the disease.

**MATERIALS AND METHODS**

**Clinical studies.** Five domestic (40263, 40264, 40265, 40266, and 40267) and one Safari (G-6-PD heterozygous; Geoffrey × domestic) (66639) cats bred at Washington State University (Pullman) were used for these studies. After baseline evaluations, the adolescent (8 to 12 weeks old) cats received 5 mg/kg methylprednisolone subcutaneously on days ~4, 0, 1, 4, 7, and 10 and 1 × 10^5 focus-forming units (ffu)/kg of in vitro-passaged FeLV-C/Sarma (in the supernatant of FEF-C cells obtained from Os Jarrett and David Onions, Glasgow, Scotland) intraperitoneally on day 0. Hematocrits, white blood cell counts, and reticulocyte indices (corrected for hematocrit) were evaluated each 2 to 3 weeks. Cats with hematocrits less than 10 or with symptoms attributable to anemia were transfused with blood from uninfected animals. This clinical protocol is identical to methods described in previous studies.10-15

Once anemia developed, the cats received suramin (obtained from the Pharmacoeconomic Resources Branch of the National Cancer Institute, Bethesda, MD), 40 mg/kg intravenously two times each week. The suramin was protected from light, diluted in saline, and filter-sterilized before use. In murine experiments, this dosage achieved serum levels (54 to 83 μg/mL) sufficient to inhibit reverse-transcriptase activity and impair retroviral infection.9 If the cat failed to respond after 3 weeks of therapy, the suramin dose was increased to 60 mg/kg, then 80 mg/kg, observing for previously described side effects (vomiting, fever, photophobia, abdominal distension, stomatitis, jaundice, dermatitis, and proteinuria).9

Previous feline studies have shown that after doses of 20 mg/kg infectious virus cannot be detected in serum with the 81-cell focus assay.4 Previous MMNC cultures have shown that after doses of 20 mg/kg infectious virus cannot be detected in serum with the 81-cell focus induction assay.21

**Marrow culture studies.** Marrow mononuclear cells (MMNC) were separated by density gradient centrifugation (1.070 g/mL; Percoll Pharmacia, Piscataway, NJ). After two washes, 0.5 to 1.0 × 10^6 MMNC were plated in 1 mL α medium (Flow Laboratories, McLean, VA) with 1.2% methylcellulose (Kodak Laboratory, Rochester, NY) supplemented with 1% bovine serum albumin (BSA; Reheis Chemical Co, Scottsdale, AZ), 20% heat-inactivated (56°C × 30 minutes) fetal calf serum (FCS; HyClone Laboratories, Logan, UT), 10% pooled normal cat serum (heat-inactivated), 10^-3 mol/L β-mercaptoethanol, penicillin/streptomycin/fungizone, and 0.5 U/mL partially purified recombinant human erythropoietin.22 After three days of incubation in a high humidity, 4% CO2, 96% air, 37°C environment, erythroid colonies (from CFU-E and containing 8 to 50 cells) were counted with an inverted microscope. After 9 to 10 days, erythroid bursts (from BFU-E and ≥250 cells) and GM colonies (from CFU-GM and > 50 cells) were enumerated.19

To determine if progenitors were unusually sensitive to heterologous complement (C'), 10^5 MMNC were incubated for 1 hour at room temperature with FCS, and then for 1 hour at 37°C with FCS or with baby rabbit serum (1:1 vol/vol; Pel-Freez, Rogers, AR) as a source of C'. The cells were washed three times and resuspended to 1 mL before 0.1 mL was plated per 1.0 mL of methylcellulose culture. The growth of BFU-E and CFU-GM in cultures of MMNC treated with C' was compared with the growth in cultures without C'.18

To assess the requirement for cat serum proteins, some cultures were established with 30% FCS, and progenitor growth under these conditions was compared with that in cultures containing 20% FCS and 10% cat serum.17 To assess the response of progenitor cells to hematopoietic growth factors, MMNC were cultured in the presence or absence of 5% medium conditioned by feline embryonic fibroblasts infected with FeLV-AG (obtained from Os Jarrett and David Onions) and concentrated (8X) by ultrafiltration (PM 10; Amicon Co, Danvers, MA) and heated to 56°C for 30 minutes to inactivate virus (FEF-A CM; previously called FEA/FeLV CM). In addition, MMNC obtained with each aspirate were cryopreserved (5 to 10 × 10^6/mL) in FCS with 5% dimethylsulfoxide (J.T. Baker Chemical Co, Phillipsburg, NJ), frozen with a controlled-rate cell freezer (Union Carbide Corp, Indianapolis, IN), and stored in liquid nitrogen. In this way culture studies could be repeated to confirm results. Although CFU-E were significantly decreased with liquid nitrogen storage and/or re thawing, the frequencies of BFU-E and CFU-GM, and their in vitro response to erythropoietin, FEF-A CM, cat serum, or C' were unchanged (references 16 and 17 and J. Abkowitz, unpublished data, 1989).

**Studies to assess retroviral infection.** Viremia was assessed by an enzyme-linked immunosorbent assay (ELISA; Leukassy, Piment-Man-Moore Company, Washington Crossing, PA). Gag-related proteins, detected by this serum assay, correlate with the presence of infectious FeLV particles.23 In some circumstances, quantitative (flu) assays for infectious retrovirus were performed by Edward Hoover, Colorado State University (Fort Collins) using 81 cells.24 To determine if feline hematopoietic progenitors were infected with FeLV, C' lysis studies were performed. For these experiments, 10^5 MMNC were incubated for 1 hour at room temperature with affinity-purified monoclonal antibodies (MoAbs) C11D8 and C15D8, which recognized distinct epitopes on gp70 (obtained from Chris Grant, Pacific Northwest Research Foundation, Seattle, WA; final concentration, 6.25 μg/mL for each antibody), and then incubated for 1 hour at 37°C with C'. The cells were then washed twice, resuspended to their original volume, and plated in methylcellulose culture. In simultaneous controls for these studies, FCS was substituted for C' or antibodies in the incubation steps. Progenitors (BFU-E, CFU-E, or CFU-GM) were considered infected if the number of their respective colonies detected after MMNC was incubated with antibodies and then C' was ≤65% of the number of colonies detected in cultures after control incubations.

Because positive results in the C' lysis assay could reflect adsorption of gp70 onto the surface of progenitor cells, rather than the synthesis of gp70 by integrated provirus and subsequent incorporation in the cell surface (ie, true infection), some studies were confirmed by indirect immunofluorescence assays of pooled erythroid bursts or GM colonies using goat antiserum directed at gag-proteins (predominantly p27; obtained from Edward Hoover). However, one concern with this latter methodology is that a positive study could reflect the de novo infection of erythroid or granulocytic cells during differentiation in methylcellulose culture and not the infection of the progenitor cell from which it originated. For this reason, cultures were performed in the presence of...
suramin (150 μg/mL). This concentration of suramin did not affect the number of progenitors detected by methylcellulose culture (data not shown), yet, in preliminary studies, it completely blocked infection of feline embryonic fibroblast cells exposed to FeLV-C/Sarma at a multiplicity of infection of 20:1 in the presence of polybrene (4 μg/mL). This approach was also used when the C' sensitivity of BFU-E made the independent evaluation of progenitor infection with C' lysis studies difficult.

RESULTS

Clinical studies. Prospective studies in representative cats are shown in Figs 1 through 4. Within 3 to 4 weeks of virus inoculation, all cats were viremic, and both BFU-E and CFU-GM were infected, as determined by C' lysis assays using MoAbs to FeLV gp70 (data not shown). In five of six cats (40263, 40265, 40266, 40267, and 66639) viremia persisted, and PRCA developed 17 to 20 weeks after FeLV-C/Sarma exposure. In contrast, cat 40264 cleared his viremia at week 6. At this time, no infectious virus was detected in the serum with the 81-cell ffl assay, and the ELISA detected only trace amounts of gag-related protein. Subsequent ELISA determinations were negative. However, both C' lysis and indirect immunofluorescence studies suggested that BFU-E and CFU-GM remained infected until week 22 (Figs 1 and 5). The cat remained healthy and did not develop anemia over the 30-week observation period. Therefore, studies obtained in this cat (Fig 1) were compared with data obtained from persistently viremic animals (Figs 2 through 4). In this way, abnormalities that...
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Fig 3. Hematologic studies from cat 40265. Beginning 4 weeks after inoculation with FeLV-C/Sarma, this cat was persistently viremic. Progenitors were infected, but BFU-E were not unusually sensitive to complement. After week 16, the frequency of CFU-E decreased, while increased BFU-E were detected. BFU-E responded poorly to FEFA CM in vitro. Anemia developed several weeks later. With suramin therapy, the frequency of CFU-E increased dramatically, although anemia continued. Similar data were obtained in studies from cat 40266. See the text for details.

correlated with viremia, with progenitor infection alone, or with anemia could be independently assessed.

The frequency of CFU-E detected in marrow aspirates from the five continually viremic cats decreased toward 0 after 14 to 18 weeks. Two to 3 weeks later, anemia developed in each cat. Consistent with our previous studies, BFU-E persisted. The frequencies of marrow BFU-E were normal (cats 40263 and 40267; Fig 2; and not significantly different from earlier values for these animals [P > .05, t-test] or from values for control cat 40264 [P > .05]) or were increased (cats 40265, 40266, and 66639; Figs 3 and 4; P values < .001). The frequencies of CFU-GM in marrow aspirates from all cats were unchanged (data not shown).

To further define the level at which erythropoiesis was impaired in the cats with PRCA, methylcellulose cultures were examined after 6 days, a time between those days when maximal numbers of CFU-E (3 days) or BFU-E (10 days) were detected. All colonies containing 50 to 250 erythroid cells were enumerated. The frequencies of these "intermediate colonies" were normal (cats 40263 and

Fig 4. Hematologic studies from cat 66639. Data from this G-6-PD heterozygous cat are similar to that for the cat in Fig 3. However, suramin therapy led to effective erythropoiesis and normalization of the hematocrit. Although the growth factor responsiveness of BFU-E returned, low CFU-E to BFU-E ratios weeks 30 through 36 suggest that some impairment of erythropoiesis remained.
40267) or increased (cats 40265, 40266, and 66639) (Table 1), suggesting that the "intermediate progenitor" is proximal to the block in erythroid differentiation.

**Studies of the sensitivity of BFU-E to complement.** BFU-E from three of six cats (40263, 40264, and 40267) were unusually sensitive to heterologous complement in the absence of antibody. This abnormality developed early, at the time that viremia developed and progenitors were initially infected (Figs 1 and 2), and thus did not predict the onset of anemia. As the complement sensitivity of BFU-E from cat 40264 persisted through week 20, the abnormality correlated with progenitor infection and not the presence of p27 gag-protein in the serum.

*Studies of the response of BFU-E to cat serum.* Baseline marrows from the five cats in which PRCA later developed had 2.2 ± 1.0 (SD) times more BFU-E and 1.4 ± 0.3 times more CFU-GM detected under culture conditions with 20% FCS and 10% cat serum versus 30% FCS alone. With PRCA (weeks 18 through 20), increments of 5.4 ± 1.9 (P < .05, paired t-test) and 1.8 ± 0.9 (P > .1) of BFU-E and CFU-GM were detected, respectively. These data confirm results from previous studies which suggest that BFU-E from cats with PRCA have altered nutrient requirements for optimal growth. Our prospective studies (representative results shown in Figs 2 and 3) failed to show a clear relationship between this finding and the onset of anemia.

### Table 1. Suramin Response in Cats With Pure RBC Aplasia

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Days of Suramin</th>
<th>Hematocrit</th>
<th>Reticulocyte Index</th>
<th>Marrow Morphology</th>
<th>CFU-E</th>
<th>Intermediate Progenitors</th>
<th>BFU-E</th>
</tr>
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<tbody>
<tr>
<td>40263</td>
<td>0</td>
<td>11</td>
<td>—</td>
<td>E = 0*</td>
<td>0</td>
<td>—</td>
<td>37 ± 81</td>
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<td></td>
<td>11</td>
<td>0.0</td>
<td>19 ± 1</td>
<td>10 ± 1</td>
<td>34 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40265</td>
<td>0</td>
<td>13</td>
<td>E:G = 1:20</td>
<td>1,096 ± 85</td>
<td>174 ± 25</td>
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<tr>
<td>4</td>
<td>7</td>
<td>0.3</td>
<td>19 ± 1</td>
<td>1,602 ± 98</td>
<td>262 ± 27</td>
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</tr>
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<td>—</td>
<td>46 ± 1</td>
<td>271 ± 26</td>
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<td></td>
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<tr>
<td></td>
<td>11</td>
<td>0.3</td>
<td>1,003 ± 22</td>
<td>676 ± 76</td>
<td>287 ± 31</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>0</td>
<td>0.0</td>
<td>E:G = 1:1</td>
<td>785 ± 57</td>
<td>314 ± 16</td>
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<td>10</td>
<td>0.1</td>
<td>E:G = 3:2</td>
<td>948 ± 57</td>
<td>330 ± 59</td>
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<tr>
<td>14</td>
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<td>—</td>
<td>256 ± 26</td>
<td>330 ± 59</td>
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<td></td>
</tr>
<tr>
<td>40267</td>
<td>0</td>
<td>11</td>
<td>E = 0</td>
<td>0</td>
<td>51 ± 5</td>
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<td></td>
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<tr>
<td></td>
<td>4</td>
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<td>0</td>
<td>70 ± 3</td>
<td>68 ± 1</td>
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<tr>
<td>11</td>
<td>11</td>
<td>0.0</td>
<td>E = 0</td>
<td>43 ± 4</td>
<td>33 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>9</td>
<td>0.0</td>
<td>E:G = 1:20</td>
<td>114 ± 18</td>
<td>188 ± 14</td>
<td></td>
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<tr>
<td>66639</td>
<td>0</td>
<td>21#</td>
<td>0.1</td>
<td>—</td>
<td>300 ± 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>26</td>
<td>0.0</td>
<td>448 ± 20</td>
<td>182 ± 12</td>
<td></td>
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</tr>
<tr>
<td>10</td>
<td>25</td>
<td>4.3</td>
<td>1,616 ± 36</td>
<td>—</td>
<td>294 ± 34</td>
<td></td>
<td></td>
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</table>

*Ratio of erythroid (E) to granulocytic (G) cells.
†Number of progenitors detected (mean ± SE) per 10⁶ marrow mononuclear cells. Erythroid colonies (from CFU-E) were counted after 3 days of culture. Intermediate erythroid colonies were enumerated at day 6 and erythroid bursts (from BFU-E) at day 10.
‡The hematocrit value reflects a recent transfusion.
§Five weeks before beginning suramin, the hematocrit was 14, and 40 ± 12 CFU-E, 739 ± 54 intermediate progenitors, and 264 ± 8 BFU-E were detected per 10⁶ marrow mononuclear cells.
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PRCA. No changes in serum requirements were noted for BFU-E (Fig 1) or CFU-GM (data not shown) from cat 40264, which cleared his viral infection and maintained a stable hematocrit.

Studies of the response of BFU-E to growth factors. When baseline marrows were cultured with 20% FCS and 10% cat serum in the presence of 5% heat-activated concentrated FEF-A CM, means of 1.9 ± 0.3 (SD) and 1.3 ± 0.2 more BFU-E and CFU-GM were detected than in cultures without FEF-A CM. BFU-E cultured at weeks 18 through 20 from all five cats with PRCA were poorly responsive to this growth factor(s) (mean increment 1.0 ± 0.1, P < .01, paired t-test), although the responsiveness of CFU-GM remained unchanged (mean increment 1.8 ± 0.9, P > .1). Stable or decreased frequencies of erythroid bursts were seen in cultures with 10% or 20% FEF-A CM (data not shown). Similar changes in the responses to growth factor(s) were seen when BFU-E and CFU-GM were cultured in 30% FCS with or without 5% FEF-A CM, consistent with our previous data. Under these serum conditions, increments of 2.7 ± 0.8 and 1.4 ± 0.3 BFU-E and CFU-GM, respectively, were seen at baseline, and increments of 1.2 ± 0.3 (P < .01, paired t-test) and 1.8 ± 0.8 (P > .1) were observed once PRCA developed.

In prospective studies in all cats (eg, Figs 2 through 4), an inappropriate response to FEF-A CM developed at the exact time that the numbers of detectable CFU-E decreased, or 2 to 3 weeks before the onset of anemia. Thus, it is likely that this finding is relevant to the pathogenesis of PRCA.

Of importance, the response to erythropoietin of BFU-E from cats with PRCA did not change. At baseline and with PRCA, plateau numbers of BFU-E were detected with 0.3 U/mL of erythropoietin and ½ plateau levels were seen at an erythropoietin concentration of 0.2 U/mL (data not shown).

Therapy with suramin. Once the anemia developed, cats were treated with suramin, 40 mg/kg intravenously, two times each week. Within 4 to 11 days, CFU-E were again detected in marrow aspirates from all cats (Table 1). In cats in which the marrow contained high frequencies of erythroid bursts and “intermediate erythroid colonies,” (40265, 40266, and 66639), the response in CFU-E to suramin was quicker and more dramatic (Figs 3 and 4; Table 1). For example, the numbers of CFU-E detected per 10⁶ marrow cells from cat 66639 (the G-6-PD heterozygous cat) increased from 0 to 1,616 ± 36 (which is over three times the baseline pre-infection levels) by 14 days. This cat then developed a reticulocytosis and corrected its anemia (Fig 4). By 21 days of suramin therapy, the number of CFU-E per 10⁶ marrow cells decreased to baseline values (435 ± 15).

After discontinuation of the drug, low frequencies of CFU-E (31 to 94/10⁶ marrow cells) and high frequencies of BFU-E (91 to 160/10⁶ marrow cells) were seen, suggesting that a partial impairment of BFU-E to CFU-E differentiation occurred. However, the hematocrit remained low normal (27 to 32) over the additional 8 weeks of observation (Fig 4). At this time the cat developed a fulminant Esch}-

The G-6-PD phenotype of progenitors from this cat was determined at baseline when anemia developed and after suramin therapy. Before infection, 39% ± 6% (SE) of BFU-E (n = 76) contained domestic (d) G-6-PD. When PRCA developed, the percent of BFU-E with d G-6-PD was unchanged (47% ± 6%, n = 78, P > .3), consistent with previous results (reference 10 and J. Abkowitz, unpublished observations, 1985 to 1990). When the anemia remitted, a similar percent of BFU-E with d G-6-PD was seen (48% ± 6%, n = 72). Therefore, the improved erythropoiesis did not result from the emergence of a neoplastic or altered clone.

The four other cats (all domestic cats) did not correct their anemia. However, there were significant although transient increases in the numbers of detectable CFU-E and hemoglobinized cells that appeared in marrow aspirates (Table 1). With increased suramin dosing (Figs 2 and 3), erythropoiesis did not further improve and the cats lost weight and became dehydrated. Postmortem examinations showed diffuse hepatocellular degeneration that suggested drug toxicity.

Characteristics of BFU-E were also studied during suramin therapy (Figs 2 through 4). The complement sensitivity of BFU-E and their requirement for cat serum for optimal in vitro growth did not change with suramin. However, an improved response of BFU-E to growth factor(s) was seen in studies of marrow from cat 66639, which chronologically correlated with the improved hematocrit (Fig 4).

When four control cats were treated with suramin (40 mg/kg two times each week for 3 weeks), no changes in hematocrit, reticulocyte index, or frequencies of CFU-E, BFU-E, or CFU-GM, or their responses to growth factor(s) were seen (data not shown).

To determine if suramin decreased the infection of erythroid progenitors by FeLV, at least transiently, leading to the improved hematopoiesis, virologic studies were performed before suramin therapy and at days 7 through 10 of suramin therapy. BFU-E and CFU-GM remained infected by C′ lysis studies using MoAbs to gp70 (data not shown). Similarly, when marrow cells were cultured in the presence of suramin at concentrations sufficient to prevent de novo retroviral infection, erythroid bursts and GM colonies contained gag-proteins with indirect immunofluorescence. Serum viral antigen levels as determined by ELISA with serial dilutions did not change (data not shown), although the 81-cell fflu assay failed to detect infectious virus, suggesting an adequate serum concentration of suramin.

These virologic data and the rapidity of the CFU-E response suggested that suramin improved erythropoiesis by a mechanism different than the inhibition of retroviral infection of progenitor cells.
DISCUSSION

Physiology of feline PRCA. PRCA is the predictable consequence of FeLV-C/Sarma viremia in cats. However, the mechanism by which this retrovirus impairs just erythropoiesis (and not granulopoiesis) and affects a specific level of differentiation (BFU-E to CFU-E) is not clear. In an attempt to correlate in vitro and in vivo observations, blood and marrow cells were studied during the time between viral inoculation (day 0) and PRCA (weeks 17 through 20). By 3 to 4 weeks, all cats were viremic and both BFU-E and CFU-GM were infected. These observations confirmed that the unique infection lysis of BFU-E was not the cause of anemia. Thus, the pathogenesis of this disease was not equivalent to that of B19 parvovirus-induced human PRCA.81

One cat (40264) cleared his systemic retroviral infection by week 6 as p27/gag-protein could no longer be detected in the serum. Yet, BFU-E and CFU-GM remained infected through week 20. This suggests that a target cell for FeLV-C/Sarma is a multipotent cell, more primitive than BFU-E or CFU-GM. Alternatively, as stromal cells are reservoirs for FeLV, low-level viral replication in microenvironmental cells during weeks 6 through 20 could result in the continued de novo infection of BFU-E and CFU-GM.

In the prospective studies of marrow from the five continually viremic cats, CFU-E frequency decreased significantly by weeks 14 through 18, 2 to 3 weeks before the onset of anemia, while BFU-E persisted. The frequencies of CFU-GM were unchanged. When the anemia developed, marrow aspirates contained infrequent erythroid cells and the reticulocyte index was < 1 (Table 1). Normal numbers of granulocytes and platelets remained. These results confirmed those of previous studies and suggested that feline PRCA resulted from the in vivo impairment of BFU-E to CFU-E differentiation. Events at this level of differentiation may be crucial to cell growth and/or the full expression of the erythroid differentiation program. It is at this stage that avian erythroblastosis virus (AEV) (which contains v-erb A and v-erb B) and Friend spleen focus-forming virus (SFFV) (via gp55) can transform avian or murine erythroblasts, respectively, to induce erythroleukemia, that the expression of receptors for erythropoietin and transferrin are upregulated, and that the physiologic regulation of the numbers of mature RBCs occurs.

Our prospectives marrow culture studies provide insights into how the gp70 of FeLV-C/Sarma impairs BFU-E differentiation. Certain in vitro abnormalities of BFU-E are likely to be epiphenomena, and not directly relevant to pathogenesis, because they had no temporal correlation with the onset of anemia. These include the rapid cell cycle of BFU-E, their sensitivity to heterologous complement, and their requirement for 10% cat serum for optional in vitro growth.

In contrast, at the exact time that CFU-E frequency decreased in marrow cultures, BFU-E no longer responded to the hematopoietic growth factor(s) present in FEF-A CM (Figs 2 through 4). This was not a relative insensitivity, as levels of FEF-A CM (20%) seven times the normal plateau concentration failed to elicit a response. Erythropoietin dose-response curves remained unchanged. Therefore, the defect in BFU-E behavior was specific, and not global. Also, the lack of response of BFU-E to FEF-A CM did not reflect the emergence of a neoplastic clone with an altered growth factor receptor, as equal numbers of erythroid bursts containing domestic or Geoffroy-type G-6-PD were seen in marrow cultures from cat 66639. As the FEF-A CM response of CFU-GM from cats with PRCA was normal, one possibility is that the number or function of receptors for growth factor(s) is significantly altered only on BFU-E (and not CFU-GM). Alternatively, distal events along a signal transcription pathway unique to BFU-E, and independent of erythropoietin, could be affected.

Suramin studies. Once PRCA developed, the cats were treated with suramin at doses sufficient to block de novo retroviral infection. In cats that had high frequencies of BFU-E (40265, 40266, and 66639), erythropoiesis quickly improved and high frequencies of CFU-E were detected within a few days (Table 1). The ratio of erythroid to granulocytic cells increased in marrow aspirates and one cat, 66639, resolved its anemia. Because reticulocyte indices remained low in cats 40265 and 40266, erythropoiesis was ineffective in these animals. Similar but less dramatic changes were seen in studies from cats that had normal frequencies of BFU-E before suramin therapy (40263 and 40267) (Table 1). Despite the improvement in erythropoiesis, BFU-E (and CFU-GM) from all cats remained infected. Therefore, suramin affected erythropoiesis through a mechanism different than the inhibition of reverse transcriptase.

Of note, suramin has diverse physiologic effects. For instance, its inhibition of lysosomal enzymes results in the systemic accumulation of glycosaminoglycans and sphingolipids. In addition, it is a potent inhibitor of the binding of platelet-derived growth factor (PDGF) to its cell surface receptor and of the intracellular binding of the v-sis gene product to the PDGF receptor. Similarly, the binding of transforming growth factor-β, heparin-binding growth factor type 2, and epidermal growth factor to their respective receptors is impaired in the presence of suramin. Thus, by analogy, if the gp70 of FeLV-C/Sarma induces disease through binding a receptor protein crucial to erythroid differentiation, this interaction could be competitively inhibited by suramin.

Hypothesis. Given these data and insights, we are pursuing the possibility that the gp70 of FeLV-C/Sarma alters interactions of transferrin with its receptor. Between BFU-E and CFU-E, the expression of the transferrin receptor is impaired in the presence of suramin. Thus, by analogy, if the gp70 of FeLV-C/Sarma induces disease through binding a receptor protein crucial to erythroid differentiation, this interaction could be competitively inhibited by suramin.
<table>
<thead>
<tr>
<th>Variable region 1</th>
<th>Variable region 2</th>
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<tbody>
<tr>
<td>FeLV gp70</td>
<td></td>
</tr>
<tr>
<td>B/Gardner-Arnstein</td>
<td>CDIQTWNPSDQEPFP----QVC-CDQPMRWWQQRNT...FYVCPGHA-----NRRCCGGPQ</td>
</tr>
<tr>
<td>A/Glasgow-1</td>
<td>CDLVOGTVEMPVIVSNVXVARYSXSRY-CTDTTRKKQXTY...FYVCPGASPWLPGKTHCGGAA</td>
</tr>
<tr>
<td>C/Sarma:</td>
<td></td>
</tr>
<tr>
<td>Transferin family</td>
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<tr>
<td>human TF</td>
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<td>pig TF</td>
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<tr>
<td>rat TF</td>
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<tr>
<td>human LTF</td>
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<td>horse LTF</td>
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<tr>
<td>mouse LTF</td>
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<tr>
<td>ovotransferrin</td>
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<tr>
<td>MetTF</td>
<td></td>
</tr>
<tr>
<td>murine LTF (C)</td>
<td></td>
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<tr>
<td>ovotransferrin (C)</td>
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</tbody>
</table>

Fig 6. A multiple alignment of a region of gp70s from three strains of FeLV compared with a multiple alignment of transferrin and transferrin homologues. The regions encompass residues 78 through 140 of the gp70s (numbering from C/Sarma) and residues 1 through 51 of transferrins (numbering from human transferrin). Asterisks (*) indicate positions in which neither FeLV-B/Gardner-Arnstein nor FeLV-A/Glasgow match FeLV-C/Sarma. Bars (I) indicate positions at which one or more members of the transferrin family matched the gp70 of FeLV-C/Sarma. A region of FeLV-C/Sarma with residues described by both (+) and (I) is indicated in bold type. Colon: (:) indicate positions at which at least one member of the transferrin family has a residue similar to that of FeLV-C/Sarma (similar residues are considered to be [D ~ E], [F ~ Y], [I ~ L ~ V], [K ~ R], [N ~ G], [S ~ T]). Dots (.) indicate gaps introduced to maintain alignment of sequences within either the FeLV group or the transferrin group. Gaps that have been introduced to maximize matches between the FeLV group and the transferrin family that correspond to positions of such intra-family gaps are also indicated by dots. Dashes (-) indicate gaps that are introduced in one group solely to maximize inter-family matches with no corresponding gap in the other family. Such an ad hoc gap appears at only one position in the alignment. Sequences are from: FeLV-C/Sarma, FeLV-B/Gardner-Arnstein, FeLV-A/Glasgow-1, human transferrin (TF), pig TF, rat TF, human lactoferrin (LTF), mouse LTF, hen ovotransferrin, melanotransferrin (MeTF), and B-lym.

**pete with apotransferrin, which weakly binds the transferrin receptor in the endosom, to affect receptor recycling. Studies with AEV suggest that an intact transferrin receptor cycle is required for subsequent hemoglobinization. Suramin then might interfere with gp70-transferrin receptor binding to allow increased binding of the true ligand, transferrin (or apotransferrin). Interactions of suramin with the transferrin receptors of the K562 and U937 cells have been described.**

Figure 6 shows a comparison between the N-terminal amino acid sequences of transferrin, lactoferrin, and other members of the transferrin family of proteins and a hydrophilic sequence from the gp70 of FeLV-C/Sarma, which encompasses variable regions 1 and 2. The envelopes of FeLV subgroup A, B, and C viruses differ in discrete areas, designated as variable regions 1 through 4 by Riedel et al. **X-ray crystallography studies show that these major regions are on the surface of lactoferrin, and thus is a potential receptor binding domain. Although homology-searching algorithms and profile analyses fail to demonstrate significant similarities in primary sequence** (11) (and Patrick O’Hara, Zymogenetics, Seattle, WA, personal communication, May 1990), we are particularly interested in potential structure/function relationships of the nine amino acids indicated by bold type in Fig 6. As aligned in Fig 6, the tryptophan residue, for instance, is uniformly conserved in the transferrin family. Murine transferrin (or apotransferrin) is also indicated by dots. Dashes (-) indicate gaps that are introduced in one group solely to maximize inter-family matches with no corresponding gap in the other family. Such an ad hoc gap appears at only one position in the alignment. Sequences are from: FeLV-C/Sarma, FeLV-B/Gardner-Arnstein, FeLV-A/Glasgow-1, human transferrin (TF), pig TF, rat TF, human lactoferrin (LTF), mouse LTF, hen ovotransferrin, melanotransferrin (MeTF), and B-lym.

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