Immune-mediated erythroid and megakaryocytic aplasia in a cat

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Case Description—A 6-month-old domestic shorthair cat was evaluated because of acute lethargy.

Clinical Findings—Severe nonregenerative anemia and thrombocytopenia were identified. Cytologic examination of a bone marrow aspirate revealed selective erythroid and megakaryocytic aplasia and a high number of apparently normal small lymphocytes. Infectious agents implicated in feline hematologic disorders were excluded on the basis of serologic tests or PCR amplification, including FeLV, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum*, and *Candidatus Mycoplasma turicensis*.

Treatment and Outcome—A 10-day course of prednisolone administration did not improve the hematologic disorder. Administration of human polyclonal immunoglobulins preceded increased reticulocyte count by 3 days. A second bone marrow examination confirmed restoration of erythroblasts and megakaryocytes. After 1 relapse, the disease was successfully controlled with prednisolone for > 3 years.

Clinical Relevance—Immune-mediated bone marrow aplasia is rare in cats and usually affects only erythroid progenitors. Concomitant involvement of erythroid and megakaryocytic cell lines can be successfully treated via immunosuppressive therapy. Human immunoglobulins seem to be well tolerated in cats; however, proof of a beneficial effect requires further study. (J Am Vet Med Assoc 2007;230:1024–1027)

A 6-month-old 2.5-kg (5.5-lb) sexually intact male domestic shorthair cat was evaluated at the Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, because of an episode of hematemesis and subsequent lethargy that occurred in the morning. The cat had appeared healthy prior to hematemesis. The cat was mostly kept indoors, regularly vaccinated, and led a commercial growth diet. On clinical examination, the cat had severely pale mucous membranes, heart rate of 120 beats/min, weak pulse, respiratory rate of 52 breaths/min, and rectal temperature of 36.0°C (96.8°F). External bleeding, skin petechiae, or ecchymoses were not observed. Other findings of physical examination were unremarkable.

On laboratory examination, severe normochromic normocytic nonregenerative anemia (Hct, 5% [reference range, 33% to 45%]; mean corpuscular volume, 41 fL [reference range, 41 to 49 fL]; reticulocyte count, 0.1 X 10³ cells/µL and marked thrombocytopenia (4 X 10³ thrombocytes/µL [reference range, 180 to 680 X 10³ thrombocytes/µL]) were detected along with adequate WBC count and differential percentages of WBCs. On a blood smear, erythrocytes, platelets, and WBCs had normal morphologic features. Results of serum biochemical tests, urinalysis, and measures of coagulation were within reference ranges. Results of serologic tests for FIV antibodies and FeLV antigen were negative. Results of fecal examination were negative for intestinal parasites.

The cat received oxygen, and blood typing and major cross-matching were performed to permit immediate transfusion of fresh whole blood (25 mL [4 mL/kg/h [1.8 mg/lb/h]]) and colloids (dextran 70, 2.5 mL/kg [1.12 mL/lb/h]) and colloids (dextran 70, 2.5 mL/kg [1.12 mL/lb/h], 1 bolus), and the cat was warmed with an infrared light. After transfusion (type A blood), the Hct increased to 11% and the clinical status improved. Subsequently, thoracic radiography and abdominal ultrasonography were performed; intracavitary bleeding or organ morphologic abnormalities were not observed. Although the cat had an episode of hematemesis, gastric bleeding was considered an unlikely cause for the severe anemia, but rather a consequence of severe thrombocytopenia and only a potential contributing factor to anemia. Chronic causes of anemia were thus more likely, and important differentials were infectious causes or a primary bone marrow disorder. The severe thrombocytopenia was suspected to be secondary to the same condition that caused anemia. On the same day, a bone marrow aspirate was collected and an aliquot was stored to perform PCR assays for FeLV, *Mycoplasma spp*, *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*), and *Ehrlichia canis*, as described.1-4

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Cytologic examination of bone marrow revealed striking hypercellularity attributable to a large number of small lymphocytes with normal morphologic features (Figure 1). An adequate number of intermediate-size lymphocytes and lymphoblasts with normal cytologic features were observed. Via scanning of multiple slides, no megakaryocytes and only rare erythroblasts were found, and more mature cell stages were absent, suggesting arrested development in both cell lines. Dysplastic changes were not evident in myeloid precursors. Disorders of the bone marrow including myelodysplasia, myelophthisis, fibrosis, and necrosis were ruled out cytologically.

Negative results of FeLV PCR assay excluded a latent FeLV infection. In addition, Mycoplasma haemofelis, Candidatus Mycoplasma haemominutum, Candidatus Mycoplasma turicensis, A phagocytophilum, and E canis genomes were not detected by use of PCR assays. By exclusion of other causes, an immune-mediated disease was suspected, oral administration of prednisolone was started (2.0 mg/kg [0.9 mg/lb], q 12 h), and prophylactic IV administration of ranitidine was performed to reduce gastric acid secretion (1.1 mg/kg [0.5 mg/lb], q 12 h). On the basis of results of blood analyses, during the first 10 days, prednisolone treatment was ineffective for improvement of the hematologic disorder (on day 10: Hct, 11%; reticulocyte count, $2 \times 10^3$ cells/µL; thrombocyte count, $10 \times 10^3$ thrombocytes/µL). At this time, human polyclonal immunoglobulins were administered IV at a dose of 1.21 g/kg (0.55 g/lb) over 6 hours without visible adverse effects. After 3 additional days, the Hct was still low (11%), but reticulocyte count had increased ($223 \times 10^3$ cells/µL). Many thrombocyte clumps were observed and prevented an absolute count; nonetheless, thrombocyte numbers appeared to have improved. Thereafter, Hct and thrombocyte counts continued to improve and returned to reference ranges 40 days after the initial diagnosis.

On the same day, examination of a bone marrow aspirate revealed normal numbers of erythroblasts and small lymphocytes (Figure 1). The number of megakaryocytes was adequate, but a subjective increase in young cells (ie, megakaryoblasts) relative to the mature stage was observed, thought to be attributable to the recent recovery. The dosage of prednisolone was slowly tapered over the successive 5 months to reach 1.3 mg/kg (0.7 mg/lb), every 48 hours. This dosage was administered for 8 weeks, when moderate nonregenerative anemia and thrombocytopenia were again observed (Hct, 19%; reticulocytes count, $0.1 \times 10^5$ cells/µL; thrombocyte count, $57 \times 10^3$ thrombocytes/µL). Higher doses of prednisolone were administered and then tapered. At 38 months from diagnosis, the cat was still in good health, and the hematologic disorder was adequately controlled with low-dose prednisolone (0.7 mg/kg [0.3 mg/lb], q 48 h).

**Discussion**

In the cat of this report, favorable outcome was achieved for a suspected immune-mediated disorder affecting bone marrow erythroblasts and megakaryocytes and associated with concomitant severe nonregenerative anemia and thrombocytopenia. On the basis of a literature search, this association has not previously been identified in cats.

Bone marrow disorders affecting blood progenitors are rare in cats. Aplasia of erythroblasts and megakaryocytes have been mostly described with FIV and FeLV infections, as well as with feline parvovirus infections. Among these diseases, FeLV infection in particular can cause selective suppression of erythropoiesis and thrombopoiesis, whereas parvovirus and FIV typically also affect myeloid progenitors or lymphoid hematopoietic cells, respectively. In addition, FeLV-infected cats with hematologic abnormalities often have dysplastic changes in blood cell progenitors. Such abnormalities were not observed in the cat reported here, and a latent form of FeLV infection was excluded by negative results of PCR assay of the bone marrow. In cats, E canis infections have also been associated with multiple hematologic disorders; in 1 cat, erythroid and megakaryocytic aplasia with corresponding bipytenia in blood was
Long-term administration of doxycycline and immunosuppressive drugs was necessary to control the disease. In the cat reported here, *E canis* and *A phagocytophilum* infections were ruled out by negative results of PCR assays of a bone marrow sample; however, infection with other *Ehrlichia* spp could not be excluded. Nevertheless, ehrlichial infection was considered unlikely because the cat had no known tick exposure. *Ehrlichia* spp are not endemic in Switzerland, and successful control of the blood disorder necessitated and was achieved with prednisolone for more than 3 years. Although not previously reported in this context, PCR assay was helpful to also exclude *Mycoplasma* spp as causative for the hematologic disorder.

Several combinations of bone marrow progenitor hypoplasias or aplasias are also found in cats with lymphoproliferative disorders that cause myelophthisis (ie, lymphoma and multiple myeloma)10; despite the reduced amount of blood-cell progenitors, invasion by the tumor increases the overall cellularity of the bone marrow, which differs from findings associated with infectious causes. Hypercellular bone marrow was also a finding in the cat reported here, which resulted from an increased population of small mature lymphocytes with normal morphologic features. Because further examination was not performed to assess whether these cells were monoclonal or polyclonal, leukemia could not be excluded at the time of diagnosis. However, leukemia was later excluded on the basis of the long-term survival by use of immunosuppressive therapy alone.

Dysmyelopoiesis is another category of diseases that affect the bone marrow and cause different types of peripheral cytopenia, including anemia associated with thrombocytopenia.6,11 Dysmyelopoiesis is a preneoplastic condition characterized by dysplastic features in >10% of 1 or several hematologic cell lines. In addition to cases caused by FeLV infection, the disorder is now increasingly recognized in cats as an idiopathic acquired disorder.11 Cytologic examination of bone marrow consistently reveals increased numbers of myeloblasts or erythroblasts, and in 15% of cats, these blast cells can be also identified in the circulation.12,13 None of these features was detected in the cat of the present report. Immune disorders of bone marrow progenitors have been rarely diagnosed in cats,14,15 and to the authors’ knowledge, this is the first description of concomitant immune-mediated erythroid and megakaryocytic aplasia. Regarding immune disorders of blood progenitors, 9 cats affected by PRCA have been reported.14 Pure red cell aplasia is a rare immune disorder characterized by severe nonregenerative anemia and absence of identifiable erythroid precursors in the bone marrow. Although the cat reported here had a different immune disorder, because erythroblasts and megakaryocytes were both affected, many remarkable similarities can be identified. Indeed, feline PRCA is consistently diagnosed at a young age (8 to 36 months). Smears of bone marrow aspirates are also frequently characterized by a high proportion of small lymphocytes, in some cases leading to hypercellular bone marrow. In addition, in cats with PRCA, as in the cat reported here, dysplastic features are not observed in any cell line, and myeloid progenitors are unaffected. Also, PRCA appears to develop slowly, resolution of the disease requires several weeks, most cats require long-term immunosuppressive treatment to maintain normal Hct, and the disease tends to recur when dosage is decreased.14 At the time the cat was admitted, laboratory tests of proven value to support an immune pathogenesis for the hematologic disorder were lacking. Specifically, the direct Coombs test in cats with primary immune hemolytic anemia had diagnostic limitations in respect to specificity;16-18 In cats with PRCA, a 50% sensitivity has been described.14 For these reasons, a Coombs test was not performed in the cat of this report. On the basis of a recent report,19 however, the diagnostic value of a Coombs test in cats with immune hemolytic anemia may be better than previously thought.19

With regard to immune-mediated destruction of megakaryocytes, a single affected cat has been described.13 In that case, hemolytic anemia and dermatitis were additional findings, and a presumptive polysystemic autoimmune disease was diagnosed. To determine the role of the immune system in thrombocytopenia and megakaryocyte disorders, in a small number of cats, rabbit anti-feline IgG antibodies conjugated with fluorescein isothiocyanate have been successfully used to identify antibodies bound to megakaryocytes.20,21 However, too few cases have so far been examined to assess the validity of the test in cats, and at that time, such a test was not available.

In humans, immune-mediated bone marrow aplasia has been rarely reported, and most cases involve 1 cell line,22 although immune-mediated erythroid and megakaryocytic aplasia has been diagnosed.15,23 Most cases are diagnosed in patients with other diseases, such as lymphoproliferative disorders, chronic infections, and polysystemic autoimmune conditions.24 In some, circulating antibodies that are selectively cytotoxic for marrow cells have been found. In others, circulating T cells suppress hematopoiesis in vitro.22 In humans, when immune disorders of bone marrow progenitors are diagnosed, the treatment of choice is immunosuppression, with prednisolone used as the first-line agent.22 In cats, corticosteroids alone or in combination with cyclophosphamide have been used successfully to treat most of the reported cats with PRCA.14 Of note, in the cat of the present report, human polyclonal immunoglobulins were added to the treatment because amelioration was not achieved within 10 days of prednisolone administration. At 72 hours after immunoglobulin administration, reticulocytes increased, followed by an increase in the Hct. Although a cause-and-effect relationship cannot be proved and the response may have simply been a delayed response to corticosteroids, the time pattern of the increasing reticulocyte count and Hct was consistent with findings in dogs25-27 and suggested a direct effect.

Polyclonal immunoglobulins may have a beneficial effect by partially attenuating monocytic activity through Fc-mediated binding, or through directly binding autoantibodies (eg, anti-idiotypic network), and by modification of complement activation.28 Similar mechanisms have been hypothesized to explain the positive influence on recovery in dogs with immune-mediated hemolytic anemia that do not respond within a week to appropriate cortico-
steroid administration. In dogs, administration of human polyclonal immunoglobulins has not been associated with acute adverse effects, even after multiple-dose treatment. In cats, data with regard to the use and safety of this product are lacking. At the authors’ institution, human polyclonal immunoglobulins are used as an adjunct treatment to corticosteroids for nonresponsive suspected immune hematologic disorders in cats. Although a beneficial effect is unproven, doses of 0.4 to 1.5 mg/kg (0.2 to 0.7 g/lb; 3 g/cat) have not been associated with the development of visible adverse effects.

References