Immune-Mediated Neutropenia in 2 Dogs

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Immune-mediated neutropenia, also termed autoimmune neutropenia (AIN), is an uncommon event, rarely documented within veterinary literature,[1,2] although the clinical profile and probable pathogenesis have been outlined in detail within medical literature.[3–5] In this paper, we review 2 different manifestations of this disorder. The 1st case primarily involved peripheral destruction of mature neutrophils, with little impact on marrow precursors, whereas the 2nd case resulted in suppression of neutrophilic granulopoiesis within marrow. In both cases, absolute neutrophil counts dropped below 200/μL.

Case 1

A 1.5-year-old 27-kg spayed female Doberman Pinscher was presented for lethargy, anorexia, and vomiting to CL's animal hospital. Five days before her illness, she had been treated with neomycin, polymyxin, and dexamethasone drops for mild conjunctivitis of the right eye. Persistence of the vomiting and anorexia over a 3-day period prompted admission of the patient for further workup.

Routine immunizations, including rabies, distemper, canine adenovirus type 2, panleukopenia, parvovirus, and leptospirosis, had been administered 4 months before presentation. Borrelia bacterin for Lyme disease had been administered 1 month later, simultaneously with the yearly heartworm check. The dog received ivermectin (10.1 μg/kg q30d).

Physical examination indicated a dog in good body condition, with a rectal temperature of 103°F and injected mucous membranes. Results of a CBC indicated mild leukopenia (3,000/μL) with severe neutropenia (150 neutrophils/μL). Neutrophils displayed moderate toxic change. Platelet concentration was slightly decreased (110,000/μL), but this result may have been spurious because it was not detected on subsequent counts. The remainder of the CBC results were within reference range. Urinalysis of a voided specimen indicated a specific gravity of 1.015, pH 7, trace protein, occasional granular casts, and rare cocci. Serum chemistry values were within reference range. Blood culture was negative. Feces were negative for parvovirus antigen. Serum titers for canine coronavirus, *Leptospira interrogans*

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(serovars *canicola, icterohaemorrhagia, grippotyphosa, hardjo,* and *pomona*), *Ehrlichia canis,* and *Rickettsia rickettsii* (Rocky Mountain spotted fever) were undetectable. Borrelia immunoglobulin G (IgG) titer was positive at >1:1,024 but presumably reflected previous Borrelia immunization. Antinuclear antibodies were <1:40. Rheumatoid factor was negative. A thyroid profile revealed T3 0.75 ng/mL (reference range 0.45–1.5), T4 1.8 μg/dL (reference range 1–4), free T4 by dialysis 19 pmol/L (reference range 6–40), T3 auto-antibodies 1.0 (reference range 0–2), T4 auto-antibodies 1.2 (reference range 0–2). Thoracic and abdominal radiographs did not demonstrate any abnormality.

Initial treatment consisted of ampicillin (22 mg/kg SC once) and enrofloxacin (2.5 mg/kg IM once). Subsequently, treatment was changed to doxycycline (3.7 mg/kg PO q24h) for 10 days, pending determination of tick-borne infectious disease serology results. Although vomiting resolved and body temperature returned to normal, CBCs over the next 2 weeks indicated persistence of the severe neutropenia. The patient developed chin and interdigital dermatitis and slight enlargement of the right popliteal lymph node. Bone marrow and right popliteal lymph node aspirates were performed.

Other than a mild lymphocytosis, the marrow evaluation was normal. Myeloid cells were in slight excess of erythroid with the myeloid to erythroid ratio (M:E) equal to 1.6:1. Maturation sequences were complete, balanced, and morphologically normal. Small mature lymphocytes accounted for 4.3% of all nucleated cells (normal for canine marrow is <1%). Iron stores were adequate. Atypical cells were not detected. Cytologic evaluation of the right popliteal lymph node was consistent with lymphoid hyperplasia.

Despite administration of antibiotics and overall improvement of attitude, the patient's dermatologic signs and severe neutropenia persisted. Severity of the neutropenia seemed disproportionate to severity of clinical signs. These observations suggested an immune component to the neutropenia, and prednisone treatment was initiated at 1.1 mg PO q12h for 2 weeks, beginning 6 weeks after presentation. Cephradine (18.5 mg/kg PO q12h) was also given for 6 weeks.

Subsequent CBCs demonstrated a rapid response to prednisone therapy. On the 1st day of prednisone therapy, neutrophils were 720/μL. By the 3rd day, the count increased to 5,600/μL. On day 15, the neutrophil count was 13,685/μL. Dermatologic signs resolved.

Prednisone was gradually tapered to 5 mg every other day. After 6 months, treatment was stopped. Two weeks later, a CBC indicated severe neutropenia, with an absolute neutrophil count of 360/μL. Mild otitis externa was detected. A 2nd bone marrow evaluation indicated mild myeloid hyperplasia, with an M:E = 3.8:1 and a moderate neutrophilic left-shift. Small lymphocytes accounted for 2.3% of all nucleated cells. An immunofluorescent antibody (IFA) test with anti-IgG and anti-C3 antibodies done at

Kansas State University on 1 of the marrow smears demonstrated positive clusters of promyelocytes.

Prednisone (1.1 mg/kg PO q24h) was administered. Neutrophil counts again rapidly increased to within reference range. Over the next several weeks, the patient began displaying Cushingoid signs, including polyuria, polydipsia, and thinning hair coat. Immunosuppression was then augmented with azathioprine, initially at 1.9 mg/kg PO q48h and then 0.9 mg/kg PO twice per week, while prednisone was very gradually decreased to 0.2 mg/kg PO q48h. Two years after the initial presentation all medications were stopped.

Approximately 1 year later, the patient was presented for her 3rd febrile episode. Physical examination indicated a mild vaginal discharge and erythematous vaginal mucosa. CBC results revealed an absolute neutrophil count of 130/µL. The patient was started on prednisone (1.1 mg/kg PO q24h), azathioprine (1.9 mg/kg PO q48h), and cephalexin (18.5 mg/kg PO q8h). Within 3 days of initiation of immunosuppressive and antibiotic treatment, the neutrophil count and the patient's temperature returned to normal.

Two months later, while still on the immunosuppressive regimen, the patient was returned because of lethargy and vomiting. CBC and serum chemistry values were within normal limits. The patient was treated with chlorpromazine (0.5 mg/kg SQ once) and dietary modification. The following morning, she was found dead. Postmortem examination revealed a perforated ulcer in the gastric body and a 1-inch piece of sharp wood in the abdomen in close proximity to the ulcer.

Case 2

A 55-kg 4-year-old spayed female Rottweiler was presented to the referring veterinarian for bilateral swelling of the carpi and tarsi, lethargy, and a mucoid vaginal discharge. Borrelia titer in serum was 1:512. *R. rickettsii, E. canis,* and antinuclear antibody titers were undetectable. Minimal laboratory data results included WBC 3,600/µL, RBC 6.54 million/µL, platelets 217,000/µL. Chemistry values were unremarkable. The dog was treated with tetracycline (22.5 mg/kg PO q8h) for 4 weeks. Lameness resolved except for the right hind leg. Attitude greatly improved.

Three months later, the dog was again presented to the referring veterinarian for lethargy, vaginal discharge, and exacerbation of the right hind lameness. *R. rickettsii, E. canis,* and antinuclear antibody titers remained negative. Borrelia titer was unchanged at 1:512. CBC results included WBC 4,400/µL, neutrophils 396/µL, lymphocytes 2,816/µL, monocytes 616/µL, eosinophils 528/µL, plasma cells 44/µL, platelets 225,000/µL. Chemistry values were within reference range. Radiographs of the hip, left stifle, and chest were within normal limits, but the right stifle contained mild degenerative changes and a mild effusion.

The patient was referred to the Veterinary Hospital of the University of Pennsylvania (VHUP), where significant physical examination findings included minimal mucoid vaginal discharge, mild cranial drawer in right stifle, chin dermatitis, and mild submandibular and right prescapular lymphadenopathy. CBC results indicated moderate leukopenia (2,800/µL), with an absolute neutrophil count of 140/µL and band count of 36/µL. Neutrophils displayed mild toxic change. The remainder of the CBC results were within

reference range. Analysis of urine collected by cystocentesis revealed SG 1,038, WBC 5–11/HPF, epithelial cells 0–2/HPF. Urine culture was positive for *Escherichia coli.* Clinical changes in the right stifle were considered consistent with a partial tear of the right cranial cruciate ligament.

The severity of the neutropenia prompted examination of a bone marrow aspirate that revealed severe neutrophilic hypoplasia and marked lymphocytosis. Erythroid precursors were in excess of myeloid precursors, with an M:E = 0.18:1, due to absence of neutrophils and neutrophilic precursors. Identifiable myeloid elements included eosinophils, monocytes, and their precursors. Atypical morphology was not detected in any of the observed cell lines. Small mature lymphocytes accounted for 39.6% of all nucleated cells. Iron stores were adequate. These findings, coupled with the clinical signs and severity of the neutropenia, were interpreted as strongly suggestive for immune-mediated pure white cell aplasia. IFA testing of marrow smears was positive for IgG and C3.

Lymph node aspirates were consistent with lymphoid hyperplasia.

Prednisone was administered at 1.1 mg/kg PO q12h. The patient was also treated with enrofloxacin (4.3 mg/kg PO q12h) then switched to ciprofloxacin (4.5 mg/kg PO q12h) for 6 weeks. The neutrophil count increased to 13,000/µL by day 10 of treatment.

Prednisone was decreased by 50% every 3 weeks until a maintenance dosage of 0.5 mg/kg PO q48h was reached. A repeat bone marrow aspirate, done 5 months later, indicated recovery of granulopoiesis and a dramatic decrease in the lymphocyte percentage. The M:E was 2.2:1, with normal morphology for all cell lines. Lymphocytes accounted for less than 1% of all nucleated cells. Despite the return to a normal marrow profile, IFA results detected IgG and C3 directed against neutrophilic elements.

Two weeks after the 2nd bone marrow evaluation, the ruptured right cruciate ligament and medial meniscus were removed, and a DeAngelis suture was placed to provide lateral stabilization of the joint. Recovery from surgery was unremarkable.

A 3rd bone marrow aspirate, performed approximately 1 year after initial presentation, revealed normal morphology and maturation of all cell lines. IFA testing was not performed on this sample.

A 4th bone marrow aspirate was obtained 4 months later. These smears contained moderate numbers of hematopoietic cells admixed with a moderate amount of loose connective and adipose tissue. All cell lines were detectable, but a valid M:E could not be determined because of poor spreading of cells within the fatty particles. IFA testing of marrow was negative for antibodies directed against neutrophils or their precursors.

The dosage of prednisone was decreased to 0.4 mg/kg PO q48h as the 1st step in an attempt to wean the dog completely from prednisone. Approximately 5 weeks later, she developed lameness in the left hind leg consistent with rupture of the left cranial cruciate ligament. The owners elected euthanasia for their dog at that time, 19 months after initial presentation to VHUP. Postmortem examination was not performed. A neutrophil count obtained 3 weeks prior to her death was 7,450/µL.

Discussion

Persistent neutropenia can be seen in association with a variety of disorders. Most commonly, neutropenia is secondary to a peripheral event, eg, severe septic suppurative inflammation, where increased needs exceed the marrow's production rate. Use of antipyretics and antibiotics may potentially compound and prolong the neutropenia by concurrent suppression of neutrophil production, although benefits of antibiotics generally outweigh their possible suppressive effects. Other potential causes of chronic neutropenia include other groups of drugs (eg, anticonvulsants, tranquilizers, antithyroid medication, phenylbutazone), sequestration within an enlarged spleen, infiltrative marrow disease, viral infection, and immune mechanisms.

Immune-mediated mechanisms, as a cause for neutropenia, are uncommon in animals, with few reports in veterinary literature.[1,2] The 2 cases discussed in this paper displayed features supportive of this diagnosis, although the precipitating events remain unknown. Both dogs demonstrated severe neutropenia disproportionate to clinical signs, positive marrow IFA test, and rapid increases in neutrophil counts after initiation of immunosuppressive doses of prednisone. In addition, dog 1's neutrophil counts decreased when corticosteroids were stopped. Dog 2 had distinctive marrow changes that included pure white cell aplasia and marked lymphocytosis.

More complete reviews of current theories of mechanisms and diagnostic strategies can be found elsewhere.[3–5] In brief, auto-antibodies are directed either at specific neutrophil antigens or against growth regulators of granulopoiesis.[6] If specific neutrophil antigens are targeted, demonstration of immunoglobulin on neutrophils or their precursors can be used diagnostically, with a sensitivity ranging from 78% to 98%.[3,4,7] Targeting of specific antigen results in opsonization of neutrophils and removal by macrophages. The actual site of removal is presumably extravascular, within spleen or marrow or both. If precursors are targeted in marrow, complete depletion of these cells, such as seen in dog 2, is possible.

In people, a distinction is made between primary and secondary autoimmune neutropenia (AIN), with the primary form described as a syndrome of infants.[8] In primary AIN patients, no associated disease or other factor is identified as a cause for the neutropenia. Patients are generally less than 3 years old. Predisposition to infections is generally mild to moderate, rarely severe. Signs commonly include infections involving skin, upper respiratory tract, and middle ear; and fevers of unknown origin. Spontaneous remission occurs within 13–20 months, although older patients are less likely to resolve spontaneously. Median neutrophil counts for patients seen at the Institute for Clinical Immunology and Transfusion Medicine, Giessen, Germany, equal 250/μL, with a monocytosis seen in 38% of patients, especially if infection is present at the time of the CBC.[3] Bone marrows are either normocellular or hypercellular with a decrease in mature neutrophils and bands.

Treatment is generally limited to intermittent use of antibiotics for control of infections. If signs mandate more aggressive therapy, temporary remissions can be achieved with large IV doses of steroids; however, prolonged use of steroids is avoided because of adverse effects in young patients.[9] Similar to humans with primary AIN, both dogs demonstrated significant improvement of clinical signs with administration of antibiotics alone but no change in neutrophil counts.

Secondary AIN is a heterogeneous disease, found primarily in older patients and frequently associated with drug exposure,[10] other immune-mediated disorders, or lymphoproliferative disease. An association with immunization has not been reported. Ages of the patients in this paper suggest secondary AIN, but the underlying causes or precipitating agents remain unknown. In dog 1, antibiotic eyedrops had been administered a few days before presentation for vomiting and discovery of the neutropenia, but whether or not the neutropenia was already present at the time of the conjunctivitis is not known. Ocular signs may have been the 1st clue that the patient was neutropenic, but the signs were not severe enough to warrant a CBC. This patient had been receiving ivermectin as a heartworm preventative. An idiosyncratic drug reaction, resulting in neutropenia, is possible, but no reports in the literature connect ivermectin with blood dyscrasias. Finally, only baseline values for tick-borne infectious agents were determined for dog 1, so the potential for AIN secondary to infection involving 1 of these agents cannot be discounted.

In dog 2, there was a significant marrow lymphocytosis, which could potentially represent a well-differentiated lymphoproliferative disorder, but other signs and the CBC did not support this interpretation. Furthermore, marked lymphocytosis was not detected in the 2nd marrow aspirate.

In summary, severe persistent neutropenia, without apparent cause, may reflect immune-mediated destruction of peripheral neutrophils or suppression of neutrophilic granulopoiesis. Evaluation of CBC data and marrow smears, and ruling out other more common causes for neutropenia, should precede initiation of immunosuppressive therapy.

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