Large granular lymphocytic leukemia in a mixed breed dog

Katherine W.M. Lau, Stephen A. Kruth, Catherine E. Thorn, William Vernau, Peter Moore

Abstract — A mixed breed dog was diagnosed with large granular lymphocytic leukemia. Immunophenotypic analysis indicated the lymphocytes were CD3+, CD8+ T cells expressing the αβ T cell receptor and a leukointegrin, α_d. Chemotherapy and splenectomy resulted in an initial reduction in the lymphocyte count.

Résumé — Leucémie lympho-granulocytaire chez un chien de race croisée. Une leucémie lympho-granulocytaire fut diagnostiquée chez un chien de race croisée. L'analyse par immunophénotypage a démontré que les lymphocytes appartenaient à la classe des lymphocytes T de type CD3+, CD8+ et qu'ils exprimaient également le récepteur αβ pour les cellules T et la leucointégrine α_d. Une réduction du comptage lymphocytaire fut notée suite à la chimiothérapie et à la splénectomie.

(Traduit par docteur Serge Messier)

Can Vet J 1999; 40: 725–728

A 9 year-old, spayed female terrier-cross that had been presented for the repair of a ruptured cruciate ligament was referred to the Ontario Veterinary College (OVC) at the University of Guelph 4 d later for evaluation of a lymphocytosis (19.6×10^9/L, reference range 1.0 to 4.8×10^9/L) and a mild nonregenerative anemia (hematocrit (Hct) 35 L/L reference range 37 to 55 L/L; erythrocyte count 4.9×10^{12}/L, reference range 5.5 to 8.5×10^{12}/L) detected on a preanesthetic complete blood cell count (CBC). She was otherwise clinically well.

The dog was bright, alert, and responsive. Abnormal findings on physical examination included lameness of the right hind limb and a positive "cranial drawer" sign. The initial diagnostic plans included a CBC, serum biochemical profile, coagulation profile (one stage prothrombin time and activated partial thromboplastin time), and urinalysis. Findings on the CBC included a marked lymphocytosis (49×10^9/L), mild neutrophilia (13×10^9/L, reference range 3.9 to 12×10^9/L), and a normal erythrocyte count (Hct 46 L/L; erythrocyte count 6.6×10^{12}/L). The lymphocytes were described as large, granular-appearing lymphocytes (LGLs). The only abnormality detected on serum biochemical profile was mild elevation of urea (11.2 mmol/L, reference range 2.1 to 9.7 mmol/L), with a normal creatinine level. The specific gravity of the urine was 1.048 and the increase in urea was attributed to dehydration. The coagulation profile was within reference range.

The differential diagnoses for the lymphocytosis included lymphocytic leukemia (acute and chronic), lymphosarcoma, and chronic infectious diseases (chronic canine ehrlichiosis). Further diagnostic plans included determining titers for *Ehrlichia canis* and *Borrelia burgdorferi*, thoracic radiographs, abdominal ultrasound, fine needle aspirate of the spleen, and bone marrow aspirate and core biopsy. The *E. canis* and *B. burgdorferi* titers were negative. Thoracic radiographs and abdominal ultrasonograms did not reveal abnormalities. Splenic cytology indicated numerous LGLs, similar in cytological appearance to those seen in the peripheral blood. However, it was uncertain if this population represented true splenic infiltration or peripheral blood contamination. Cytological examination of the bone marrow revealed the presence of normal marrow cell lines and a normal myeloid to erythroid ratio, with synchronous and complete cellular maturation. No LGLs were detected; however, plasma cell hyperplasia was observed. Histopathological examination of the bone marrow core biopsy confirmed a normal myeloid to erythroid ratio with synchrony of cellular differentiation. The iron stores were adequate and there was no evidence of a neoplastic cell population.

The dog was presented again 6 d later and reported to have been inappetent for the last few days. No abnormalities were noted on physical examination. A CBC showed a further increase in the lymphocyte count (77×10^9/L). Flow cytological examination was performed by using rat anti-canine CD8 and rat anti-canine CD4 (Serotec, Oxford, UK) monoclonal antibodies for T cell analysis. The B cells were incubated with sheep serum to prevent nonspecific binding of the monoclonal antibody, and were subsequently stained with 1:20 dilution of fluorescein isothiocyanate-labeled sheep anti-canine IgG (heavy and light) (Serotec). The results indicated that the lymphocyte population was 97% T cells (normal range 46% to 72%) and 2.9% B cells (normal range 7% to 30%). The T cell population consisted of 98.3% CD8+ (T cytotoxic/suppressor) cells and 5.0% CD4+ (T helper) cells. A diagnosis of malignancy of CD8+ T lymphocytes (T cell leukemia) was made.

Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2A4 (Lau, Kruth); Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia, USA (Thorn); Department of Pathology, Microbiology and Immunology, University of California, Davis, California USA (Vernau, Moore).

Address correspondence and reprint requests to Dr. Stephen A. Kruth.

Table 1. Hematological results during chemotherapy in a dog with large granular lymphocytic

Days	Therapy	Hct L/L	RBC $\times 10^{12}$/L	Platelet $\times 10^9$/L	WBC $\times 10^9$/L	Neutrophil $\times 10^9$/L	Bands $\times 10^9$/L	Lymphocyte $\times 10^9$/L	Monocyte $\times 10^9$/L	Eosinophil $\times 10^9$/L
−4		34	4.9	Normal	28.8	7.8	—	19.6	1.2	0.3
0		46	6.6	242	64.2	13	0.64	49	0.64	0.64
8		45.9	6.47	169	90.9	9.1	—	77	1.8	0.91
10	Doxorubicin	44.6	6.33	245	87.9	13	—	72	0.88	0.88
17		38.6	5.63	211	75.2	1.2	—	72.19	1.5	0
25	L-asparaginase	39.3	5.75	331	90.1	11.7	0.9	73	1.8	0
28	Vincristine	40	5.79	352	114.5	6.87	—	103.05	1.15	2.29
32		35.7	5.24	217	46.6	3.3	—	42	0.47	0.47
35		37.1	5.46	178	57.7	1.7	—	50	1.2	0.58
42	Cyclophosphamide + Prednisone	36.4	5.29	385	78.8	38	1.6	38	0	0
49	Doxorubicin + Prednisone	35.4	4.97	281	42.3	11	—	30	0.85	0
56	Vincristine	34	4.85	169	53.6	11	—	41	2.2	0
70	Cyclophosphamide	41.6	5.97	339	61.7	15	0.62	42	0.62	1.9
77	Doxorubicin	38.9	5.09	282	47.6	6.7	0.48	39	0.48	0.48
85	Splenectomy	40.7	5.76	215	43	3.5	—	38	0.86	0.43
86		37.2	5.35	202	37.5	4.9	25	18	2.2	0
88		32.6	4.51	336	71.8	25	3.6	37	3.6	0
95		37.3	5.11	466	54	17	—	32	1.6	0
116		41.9	6.22	461	81.4	13	—	64.3	3.3	0.81
148		41.4	5.96	452	125.3	10.02	—	110.26	2.51	2.51
396		32	4.9	390	116.7	7.002	—	105.03	3.5	1.167
620		33	4.8	283	221	13.26	—	205.53	2.21	—

Hct — hematocrit; RBC — red blood cell; WBC — white blood cell

With the rapid increase in lymphocyte count, the clinical signs of inappetence, and the concern for splenic infiltration, the disease appeared to be behaving like an "acute" leukemia. Combination chemotherapy with L-asparaginase, vincristine, cyclophosphamide, doxorubicin, and prednisone was administered for 6 wk. Serial CBCs revealed an initial decrease in the lymphocyte count; however, normalization of the lymphocyte count was not attained during this induction period (Table 1).

During the period of chemotherapy, the dog was alert but quieter than normal and lame only after exercise. Abnormal findings on physical examination on Day 85 included a generalized increase in normal lung sounds and splenomegaly. Diagnostic tests included a CBC and serum biochemical profile, which revealed a lymphocytosis (38×10^9/L) and a mild increase in alanine aminotransferase activity (175 U/L, reference range 0 to 130 U/L). A bone marrow aspirate and core biopsy and thoracic and abdominal radiographs were performed. Radiographic abnormalities included a mild generalized interstitial pulmonary pattern and moderate splenomegaly. Cytological examination of the bone marrow aspirate confirmed an increased number of plasma cells, but no neoplastic cells were noted. Microscopic examination of the bone marrow indicated myeloid hyperplasia with evidence of early asynchrony and a decrease in the marrow granulocyte reserve. No neoplastic cells were detected.

Canine leukocyte antigen analysis, using a monoclonal antibody (MAb) panel, indicated a population of 0.48% CD21+ B cells (MAb CA2.D6 and CA2.5G2) and 97% CD3+ T lymphocytes (MAb CA17.2A12). The T cell population contained approximately 2% CD4+ T helper cells (MAb CA13.1E4) and 95% CD8+ T cytotoxic/suppressor cells (MAb CA9.JD3 and CA15.4G2). Ninety-five percent of CD8+ cells expressed the β2 integrin, α_d (MAb CA11.8H2), and αβ T cell receptor (MAb CA15.8G7). All the above monoclonal antibodies were supplied by one of the authors (PM). A diagnosis of T cell leukemia was further refined to LGL leukemia of CD3+ cytotoxic T cells expressing the αβ T cell receptor (TCR) and the novel leukointegrin, α_d.

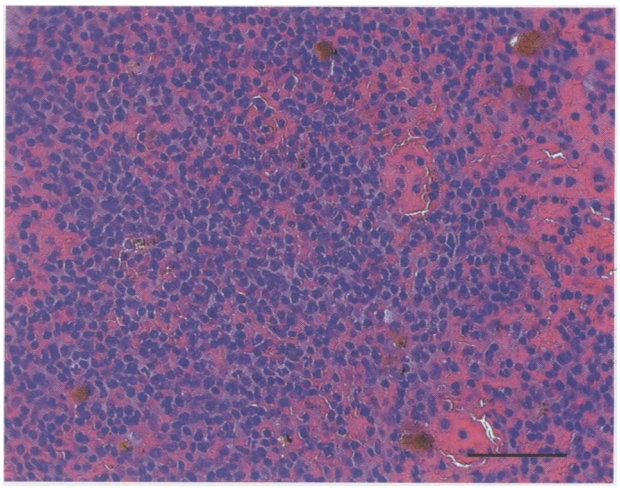

Figure 1. Photomicrograph of the spleen showing the red pulp heavily infiltrated with a monomorphic population of mature appearing lymphocytes that have relatively small nuclei and clumped chromatin. The red pulp medullary chords are predominantly affected but there is some invasion into the red pulp sinuses as well. Hematoxylin and eosin stain. Bar = 100 μm.

A laparotomy, a hepatic biopsy, and a splenectomy were performed. Microscopic examination of the spleen revealed significant infiltration of LGLs in the red pulp (Figure 1). The red pulp chords were predominantly

involved, but there was also sinus invasion. Additionally, marked white pulp atrophy, likely secondary to chemotherapy, and some splenic extramedullary hematopoiesis was noted. The hepatic biopsy showed hypercellularity around the portal areas, consisting of a mixed mononuclear cell population with occasional neutrophils. No evidence of neoplastic cellular infiltrates were found in the liver.

Postoperatively, the dog was bright and her appetite improved. Complete blood cell counts were evaluated every 1 to 6 mo, and showed a gradual increase in lymphocyte counts (205×10^9/L). Follow-up chemotherapy was not administered, as the dog remains clinically normal.

Large granular lymphocytes are characterized by the presence of abundant cytoplasmic azurophilic granules (1). They are a unique population of lymphocytes that normally comprise approximately 3% to 5% of the normal peripheral blood lymphocytes. There are 2 subsets of LGL: a T cell lineage that expresses the CD3/ TCR complex, and a natural killer (NK) cell lineage that mediates non-major histocompatibility complex (MHC) restricted cytotoxicity and does not express the CD3/ TCR complex (1,2). Lymphocytosis with LGL can be classified according to the etiology of the LGL: (1) reactive lymphocytosis and (2) malignant transformations, known as LGL leukemia (2,3). Reactive lymphocytosis occurs secondary to infectious diseases and chronic immunologic stimulation (3). In dogs with ehrlichiosis, lymphocytosis with LGL has been reported; it resolved following appropriate antimicrobial therapy (4).

Large granular lymphocytic leukemias are defined as LGL proliferations that are clonally derived from either CD3+ or CD3− LGL. The classification of T cell LGL (CD3+) or NK (CD3−) LGL is used in humans to differentiate likely prognostic outcome and therapeutic implications for each subset of disease (2). In the few dogs in which surface markers have been evaluated, CD3+ LGL have also expressed CD8, suggestive of a cytotoxic T cell origin (1). It has been demonstrated that LGL leukemia in dogs may arise from diverse lineages of lymphocytes, including 60% with αβ TCR and 32% with γδ TCR expression (5). Natural killer (CD3−) LGL leukemias have been found in 8% of LGL leukemias in dogs and is also a rare disease in humans (2,5).

The dog in this case report had CD3+ LGL leukemia of cytotoxic T cells (CD8+) with expression of αβ TCR and a novel leukointegrin, known as α_d. The canine leukointegrin α_d, described by Danilenko et al (6), was found to be expressed predominantly in macrophages of splenic red pulp, lymph node medullary regions, bone marrow, and in a minor population of CD8+ T cells in the peripheral blood (including LGL) and spleen (6). Unfortunately, in this case, leukocyte antigen analysis was not performed on the spleen to ascertain the origin of the LGL, but with the significant infiltration of lymphocytes found on microscopic examination, the spleen was likely the primary site. The presence of canine α_d has also been noted in 92% of canine LGL lymphocytoses (5,6). However, its prognostic implication is unknown at this time.

In humans, T cell LGL leukemia is more commonly reported in middle to older age individuals. The affected people often develop recurrent bacterial infections, secondary to severe neutropenia, and, occasionally, pure red cell aplasia and an increased incidence of rheumatoid arthritis (1,2,7). Pathologically, splenic, hepatic, and bone marrow infiltrations have been reported and a few cases show lymphomatous infiltration of the small intestines (2). Plasmacytosis of splenic red pulp and marrow were frequently detected (6). The histopathological findings in this dog included evidence of plasmacytosis of the marrow and, on follow-up biopsy, a reduction in the marrow granulocyte reserve. However, neutropenia, unrelated to chemotherapy induced myelosuppression, was not documented during serial hematological examinations.

The mechanisms of the neutropenia in humans are believed to be associated with a "maturation arrest" and an immune-mediated destruction of the myeloid series in the marrow (2,7). The identification of severe neutropenia is the primary indication for treatment with chemotherapy (low dose methotrexate, chlorambucil, cyclophosphamide, or prednisone), with partial and complete remissions having been reported (2). Splenectomy has been used in aggressive cases that were unresponsive to chemotherapy. Only limited success was reported in correcting neutropenia and an increase in the numbers of LGL leukemia cells was seen in some cases (2,8). However, in chronic lymphocytic leukemia (CLL) in humans, splenectomy was reported to increase survival time of patients with aggressive disease, with some cases resulting in complete remission (9).

Large granular lymphocytosis has been reported in 3 middle-aged and older, large breed dogs, but leukocyte antigen analyses were not reported in these cases (10). All 3 dogs had leukocytosis characterized by marked lymphocytosis; moderate anemia, mild to moderate thrombocytosis, and neutropenia were detected terminally in 2 of the cases. One dog was treated with chemotherapy (protocol undisclosed) and attained clinical and hematological remission for 82 d, then subsequently succumbed to the disease. The second dog was euthanized 5 d after presentation, following lack of clinical response to low-dose cytosine arabinoside. The third dog had a more chronic clinical course and achieved hematological remission for 18 mo with multiple agent chemotherapy (protocol undisclosed). It was treated prior to the development of anemia and neutropenia, and its lymphocytosis was relatively lower than in the other cases. This dog also demonstrated LGL with surface markers for crystalline fraction (Fc) portion of gamma immunoglobulins (IgG) and, therefore, may have represented a subset of T lymphocytes (10). In our present case, due to the rapid increase in LGL over 10 d, treatment with multiple chemotherapy was attempted, but only partial remission was attained. The ineffectiveness of chemotherapy may be associated with the indolent nature of the disease or the expression of multidrug resistance (MDR) genes by LGL leukemia cells, as demonstrated in LGL in humans (11).

In this case, the initial rapid increase in LGL cells, presence of early asynchrony of the myeloid to erythroid

ratio, and reduction in granulocyte reserve in the marrow were suggestive of ongoing disease. Splenectomy was performed as the spleen was suspected to be the origin of the LGL. The surgery was performed 8 d after doxorubicin therapy, which was during the expected nadir (7–10 d). However, the dog did not show significant myelosuppression following the initial doxorubicin treatment and a CBC on Day 85 had revealed an adequate neutrophil count. The decision not to delay surgery was based on the minimal myelosuppression noted following doxorubicin therapy, and the owners had requested that the surgery be performed as soon as possible, as they were planning to travel abroad.

Traditionally, T cell LGL leukemia in humans has been reported to be an indolent disease that did not require treatment. However, a recent study showed that the majority of patients had chronic disease with significant morbidity and mortality (36% died over 2 y) due to severe neutropenia (2). Poor prognostic indicators included rapid doubling time of LGL, infiltration of multiple organs, and the development of hematological abnormalities. Some patients developed an acute aggressive form that did not respond to combination chemotherapy (2).

The subset of NK lineage LGL leukemia has been reported to be more aggressive in humans, and analysis of leukocyte antigens is necessary to determine the nature and to predict the course of the disease. Hematologically, neutropenia is mild, but anemia and thrombocytopenia are more pronounced (2). Rapid increases in LGL can occur over a few weeks and severe hepatosplenomegaly is common. A few people have an initial chronic phase of disease for several years prior to developing fulminant disease (2).

The true prevalence of T cell LGL leukemia in dogs is unknown, as cases may not be presented for evaluation unless clinical signs of hematological abnormalities (neutropenia, anemia, or thrombocytopenia) or infiltrative disease are discovered. The use of leukocyte antigen analysis is important for identification of the subsets of LGL leukemia and the development of therapeutic approaches. In this case, the use of chemotherapy may have resulted in a delay in the development of severe neutropenia and infiltrative disease, reported in other cases. The initial rapid increase in LGL cells, presence of early asynchrony of the myeloid to erythroid ratio, and reduction in granulocyte reserve in the marrow were suggestive of ongoing disease. It is uncertain if splenectomy played a beneficial role in the treatment of this dog. A number of different disorders appear to be encompassed by LGL leukemia, so that the therapeutic indications and the prognosis are currently unknown in dogs. CVJ

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