NOTE *Clinical Pathology*

Eosinophilia and Eosinophilic Infiltration into Splenic B-Cell High-Grade Lymphoma in a Dog

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(Received 3 December 2009/Accepted 27 April 2010/Published online in J-STAGE 11 May 2010)

ABSTRACT. A 13-year-old mixed-breed dog showing ascites, anorexia and anemia was found to have leukocytosis with marked eosinophilia, splenomegaly and hepatomegaly. The dog died 4 days after initial presentation and was diagnosed with splenic high-grade B-cell lymphoma at necropsy. Remarkable infiltrations of eosinophils were observed in spleen and liver tissues. The eosinophilia and infiltration of eosinophils into the lesions could have been associated with B-cell lymphoma because causes other than lymphoma were excluded. This is the first report of eosinophilia and eosinophilic infiltrations into neoplastic lesions in a dog with high-grade B-cell lymphoma.

KEY WORDS: B-cell centroblastic lymphoma, canine, hypereosinophilia, infiltration of eosinophils.

J. Vet. Med. Sci. 72(10): 1367–1370, 2010

Eosinophilia can occur in dogs with non-neoplastic diseases such as allergy, parasitism, pulmonary eosinophilic infiltration and eosiniphilic gastroenteritis [6]. In addition, neoplasia-associated eosinophilia in dogs has been reported in the case of both hematopoietic and nonhematopoietic tumors, including anaplastic mammary carcinoma [7], oral fibrosarcoma [3] and rectal adenomatous polyps [14]. Among hematopoietic tumors, mast cell tumors, eosinophilic leukemia and lymphomas can induce eosinophilia [6].

Lymphoma is broadly divided into two major groups, B-cell and T-cell, on the basis of the immunophenotype of neoplastic cells. Hypereosinophilia and eosinophilic infiltration have been reported in dogs with intestinal T-cell lymphoma [8, 11]. However, neither eosinophilia nor eosinophilic infiltration has been reported previously in dogs with B-cell lymphoma. The present report describes the case of a dog with splenic high-grade B-cell lymphoma presenting with eosinophilia and eosinophilic infiltrations into neoplastic lesions.

A 13-year-old spayed female mixed-breed dog was referred to the Veterinary Medical Center of the University of Tokyo with a 1-month history of anorexia and ascites and a 2-week history of anemia. On physical examination, the dog was found to be emaciated (Body Condition Score: 2/5), the abdomen was distended and the visible mucous membranes were slightly pale. No surface lymph node swelling was observed, and there was no evidence of dermal parasites or dermatitis.

A complete blood cell count indicated nonregenerative anemia and leukocytosis with marked neutrophilia and eosinophilia (Table 1). In a peripheral blood smear, an increase of mature eosinophils was noted (Fig. 1A and 1B). Blood chemistry revealed hypoalbuminemia and a slightly increased concentration of C-reactive protein (CRP). The coagulation profile showed a slightly prolonged activated partial thromboplastin time (aPTT; Table 1). A heartworm antigen test (Snap Heartworm Antigen RT; IDEXX, Westbrook, ME, U.S.A.) was negative. A fecal flotation was negative for intestinal parasites.

Radiographic and ultrasonographic examinations of the thoracic and abdominal regions revealed pleural effusion, ascites, splenomegaly and hepatomegaly. In addition, multiple hypoechoic lesions were observed in the spleen, and mild mitral regurgitation was noted. The pleural effusion and ascites were clinicopathologically determined to be modified transudates, and cytology showed abundant mature eosinophils as well as neutrophils and macrophages without bacteria (Fig. 1C and 1D).

Although a splenectomy was planned to establish a diagnosis, the dog developed anuria the next day. Laboratory profiles revealed anemia, thrombocytopenia, hypoglycemia, azotemia, increased concentrations of serum creatinine and CRP, prolonged aPTT and elevation of fibrin/fibrinogen degradation products. These findings indicated that the dog clinically presented with disseminated intravascular coagulation and acute renal failure. The dog produced urine temporarily following a blood transfusion, an intravenous infusion with dalteparin (100 U/kg/day) and dopamine (5 μg/kg/min) and intravenous administration of furosemide (started at 1 mg/kg and increased gradually until the patient responded). However, anuria was observed again on Day 3, and the dog died on Day 4. During hospitalization, the count of eosinophils in the peripheral blood continued to increase to 22,200/μl.

Necropsy revealed duodenal perforation, ascites, splenomegaly and hepatomegaly. The mediastinal, tracheo-

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Table 1. The results of complete blood cell count and blood chemistry at the Days 1, 2 and 4

	Day 1	Day 2	Day 4
RBC ($\times 10^6/\mu l$)	3.41	2.72	3.19
Hemoglobin (g/d*l*)	8.1	6.3	7.5
PCV (%)	23	17	24
WBC (/μl)	70,800	53,500	62,000
Neutrophils, band (/μl)	5,000	3,700	3,100
Neutrophils, segmented (/μl)	39,600	14,500	27,500
Eosinophils (/μl)	14,900	17,120	22,200
Lymphocytes (/μl)	2,800	8,500	4,300
Monocytes (/μl)	8,500	5,400	4,900
Basophils (/μl)	0	0	0
Platelets ($\times 10^3/\mu l$)	544	136	158
BUN (mg/d*l*)	36.3	75.9	100.7
CRE (mg/d*l*)	0.7	3.7	4
ALP (U/*l*)	333	541	2,442
ALT (U/*l*)	32	528	898
ALB (g/d*l*)	2.7	2.4	2.4
T-Cho (mg/d*l*)	207		
Ca (mg/d*l*)	9.8	10.2	9.7
P (mg/d*l*)	3.9	11.7	19
Na (mEq/*l*)	147	146	151
K (mEq/*l*)	3.7	5.5	2.9
Cl (mEq/*l*)	109	110	113
CRP (mg/d*l*)	2.75	8.8	4.9
PT[a)] (sec)	8.5	9.5	
aPTT[b)] (sec)	25	40.8	
FDP[c)] ($\mu g/\mu l$)	< 2.5	40	

a) Prothrombin time. b) Activated partial thromboplastin time.
c) Fibrin/fibrinogen degradation products.

bronchial and mesenteric lymph nodes were swollen. Multiple anemic infarcts were observed in the spleen and kidneys, and many white spot lesions were found on the surface of the liver. Histopathologic examinations revealed proliferation of malignant lymphoid cells in the spleen (Fig. 1E), infiltration of the malignant cells into the liver (Fig. 1G) and the lung and intravascular microthrombi in the lung. The lesions in the liver were determined to be invasions because the proliferation of the malignant cells and the infiltration of eosinophils were observed multifocally in the sinusoid. Examination of the intestine showed necrosis and infiltration of fibrins and neutrophils in the greater duodenal papilla. The neoplastic cells showed no metachromatic property by staining with toluidine blue. The morphologic features of the neoplastic cells were as follows: they were medium to large in size, had narrow and basophilic cytoplasm, a round nucleus and fine chromatin patterns and most cells had a few nucleoli in the margin of the nucleus, while others had a nucleolus in the middle of the nucleus (Fig. 1F). Marked infiltration of eosinophils was also observed in the spleen and liver (Fig. 1H and 1I).

Immunohistochemical examinations were conducted using goat anti-dog IgG-h&l antibody (dilution, 1:200; Bethyl, Montgomery, TX, U.S.A.), rabbit anti-CD3 antibody (1:50; Dako, Kyoto, Japan), rabbit anti-CD117 antibody (1:400; Dako) and mouse anti-Mast cell tryptase (1:100; Dako) as primary antibodies. The tumor cells were positive for dog-IgG (Fig. 1J) and negative for CD3 (Fig. 1K), CD117 (Fig. 1L) and Mast cell tryptase (Fig. 1M).

Genetic clonality was assessed by polymerase chain reaction with heteroduplex analysis using DNA extracted from the splenic lesion as described previously [2, 15]. A clonal rearrangement was observed at the expected size (120 bp) for *IgH* major, whereas no clonal rearrangement of *TCR*γ was detected using specific primer sets [2]. Consequently, the lesion was diagnosed as splenic high-grade B-cell lymphoma and was classified as a centroblastic polymorphic type based on the updated Kiel classification [4].

Bone marrow aspiration at necropsy revealed slight hypercellularity, a decrease in segmented neutrophils and megakaryocytes and a slightly low myeloid/erythroid ratio (0.79; Fig. 1N and Table 2). In addition, malignant lymphoid cells were observed sporadically.

The causes of eosinophilia in dogs generally include allergy, parasitism, pulmonary infiltrates with eosinophils, eosinophilic gastroenteritis and neoplasms such as mast cell tumors and lymphoma [6]. In the present case, all of these causes except lymphoma could be excluded. Clinical signs of skin or respiratory apparatus were not present, and neither endoparasites nor ectoparasites were observed. The patient received no drugs other than furosemide during the 3 months before diagnosis. The maturity of the eosinophils in the peripheral blood made eosinophilic leukemia unlikely, and the necropsy showed no evidence of eosinophilic leukemia in the bone marrow. Based on these findings, the hypereosinophilia of the dog could have been caused by splenic lymphoma.

Immunohistochemistry results and genetic analysis confirmed that the immunophenotype of the lymphoma in the dog was the B-cell type. Thus far, neither eosinophilia nor infiltration of eosinophils has been reported in dogs with B-cell lymphoma, although both have been reported in dogs with T-cell intestinal lymphoma [8, 11]. In human medicine, paraneoplastic eosinophilia is often observed in patients with lymphoma. Approximately 15% of Hodgkin lymphoma cases and approximately 5% of non-Hodgkin lymphoma cases present with eosinophilia [13]. In addition, eosinophilia and infiltration of eosinophils are reported to be rare in B-cell lymphoma [10, 12, 13, 16].

In human B-cell lymphoma with paraneoplastic eosinophilia, the eosinophilia is thought to be caused by the production of interleukin-5 (IL-5), IL-3 and granulocyte-macrophage colony-stimulating factor by B-cell lymphoma cells or non-neoplastic T lymphocytes activated by B-cell lymphoma cells [13]. IL-5 is a major soluble factor for mediating eosinophilia, and the production of IL-5 by T-cell lymphoma cells has been reported previously [1, 5, 9]. Quantifying the expression of such cytokines at the mRNA or protein level could clarify the cause of the eosinophilia, but it could not be examined in the present case. However, it may be conceivable to think that the eosinophilia in this case was paraneoplastic because the possible causes except

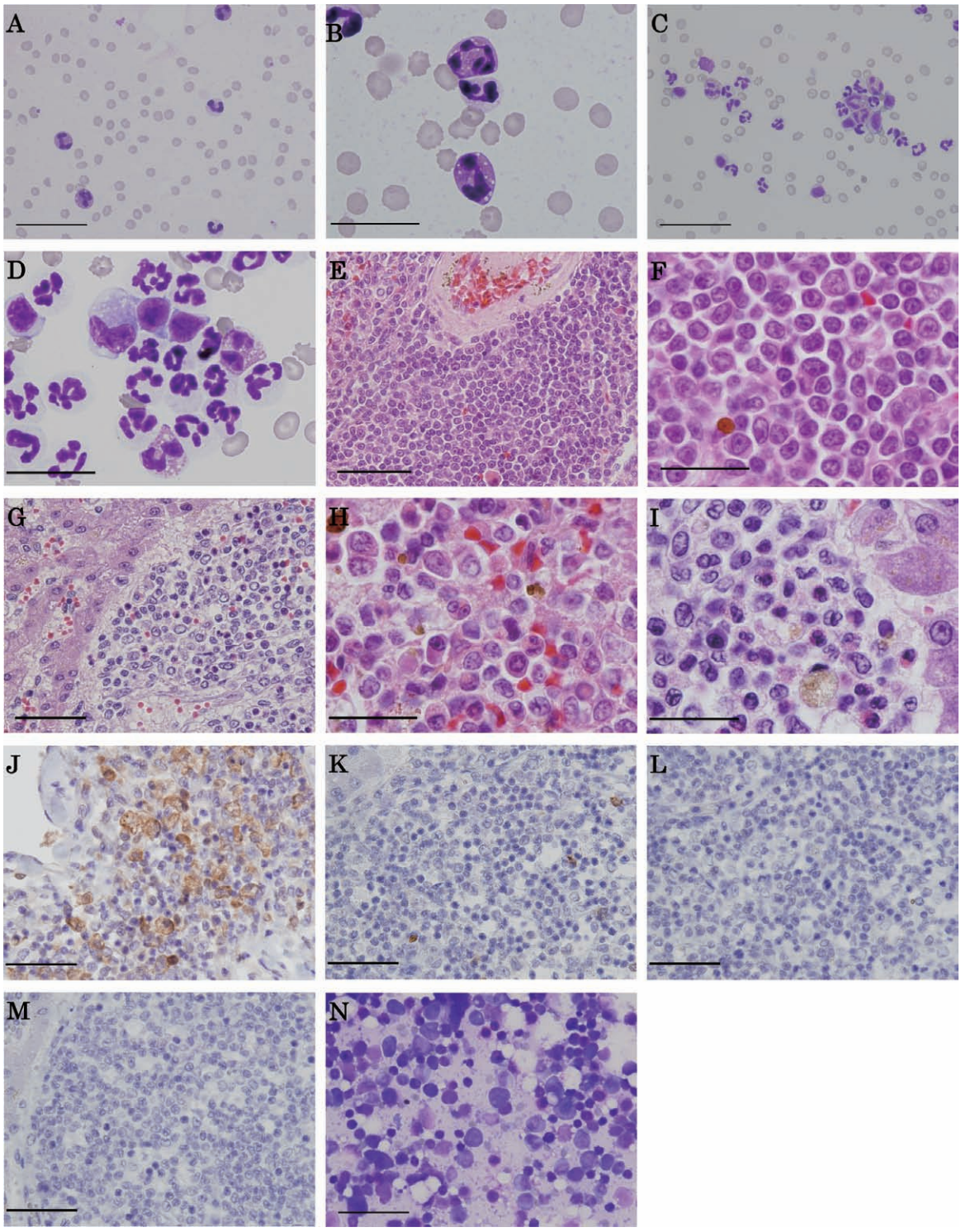

Fig. 1. Cytological findings of the peripheral blood, ascites and bone marrow smears and histopathological and immunohistochemical findings of spleen and liver. Increased mature eosinophils in peripheral blood (A, B) and ascites (C, D) are observed. In the spleen, malignant lymphoid cells proliferate diffusely (E) and are morphologically classified as centroblastic polymorphic lymphoma (F). In the liver, focal infiltrations of lymphoma cells are observed (G). In addition, infiltrations of eosinophils are observed in both the spleen (H) and liver (I). The lymphoma cells are positive for IgG (J) and negative for CD3 (K), CD117 (L) and mast cell tryptase (M). There was no evidence of eosinophilic leukemia in bone marrow at autopsy (N). A, B, C, D and N are stained with Wright-Giemsa. E, F, G, H and I are stained with hematoxylin-eosin. J, K, L and M are counterstained with hematoxylin. Bar=25 μm (B, D), 50 μm (A, C, F, H, I and N) or 100 μm (E, G, J, K, L and M).

Table 2. Myelograms by bone marrow cytology at autopsy

Proerythroblasts	3.7%
Basophilic erythroblasts	8.0%
Polychromatic erythroblasts	17.0%
Orthochromatic erythroblasts	15.3%
Myeloblasts	1.0%
Promyelocytes	2.0%
Neutrophilic myelocytes	3.7%
Neutrophilic metamyelocytes	6.3%
Neutrophils, band	10.0%
Neutrophils, segmented	8.0%
Eosinophilic myelocytes	0.3%
Eosinophilic metamyelocytes	0.7%
Eosinophils, band	1.0%
Eosinophils, segmented	1.7%
Monocytes	4.0%
Lymphoblasts	8.3%
Lymphocytes	6.0%
Plasma cells	2.3%
Megakaryocytes	0.7%
Myeloid/Erythroid ratio	0.79

lymphoma were eliminated, as described above.

This is the first report of a dog with splenic B-cell high-grade lymphoma showing eosinophilia and eosinophilic infiltration into neoplastic regions. Because the dog died on Day 4, the response to treatment and prognosis are unclear for such cases. Therefore, more of such cases need to be studied.

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