A Case of Atypical Canine Lymphoma with Oral Mass and Multiple Osteolysis

Tetsuro ITO¹⁾, Masaharu HISASUE²⁾, Sakurako NEO²⁾, Takefumi ISHIKAWA²⁾, Takuo SHIDA³⁾, Toshifumi WATANABE¹⁾, Yoshitaka ENOMOTO⁴⁾, Masaru OKUDA⁵⁾, Ryo TSUCHIYA^{2)*}, Hiroo MADARAME¹⁾, Munetsugu OGATA¹⁾ and Takatsugu YAMADA²⁾

¹⁾Veterinary Teaching Hospital, ²⁾Laboratory of Veterinary Internal Medicine II, and ³⁾Department of Veterinary Radiology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagami-hara, Kanagawa 229-8501, ⁴⁾Enomoto Animal Hospital, 6-5-13 Akikawa, Akiruno, Tokyo 197-0804 and ⁵⁾Faculty of Agriculture, 1677-1 Yoshida, Yamaguchi University, Yamaguchi-shi, Yamaguchi 753-8515, Japan

(Received 5 January 2007/Accepted 18 May 2007)

ABSTRACT. An 8-year-old female Golden Retriever had an oral mass and lameness. Multiple osteolysis of the systemic skeleton without monoclonal gammopathy was shown on electrophoresis of serum and urine samples. Cytological and histopathological examinations of the oral mass revealed atypical polymorphic cells similar to myeloid cells, and bone marrow aspiration indicated that these abnormal cells also might have invaded the bone marrow. These cells were negative to peroxidase and non-specific esterase staining, and clonal expansion of B lymphocytes could be detected by polymerase chain reaction (PCR) assay for antigen receptor gene rearrangement. The case was diagnosed as atypical lymphoma and treated by multi-drug chemotherapy. On the 142nd day after the first admission, the case had remission and the oral mass and multiple osteolysis were improved.

KEY WORDS: canine, lymphoma, PCR.

J. Vet. Med. Sci. 69(9): 977-980, 2007

Canine lymphoma is defined as a lymphoid neoplasm that originates in solid hematological organs including the lymph nodes, liver, spleen, skin, central nervous system, and eyes [5, 13]. Lymphoma is one of the most common canine neoplasms, accounting for 5-7% of all canine tumors and 85% of canine hematological tumors [5, 13, 14]. The multicentric form is the most common type and accounts for more than 80% of all cases; however, oral masses are uncommon in lymphoma [13, 14]. The tumor cells are often composed of round nuclei and cytoplasm like mature or immature lymphocytes. However, a few cases show atypical forms, like monocytic cells. In most cases, the diagnosis of canine lymphoma is made by cytological and histopathological examinations. Recently, Burnett *et al.* reported that analysis of the multiple morphology of lymphocyte antigen receptor genes may be a useful tool for determining the clonality of lymphocytes [1, 3]. We diagnosed a case of canine atypical lymphoma with an oral mass and multiple osteolysis by using clonal analysis.

An 8-year-old female Golden Retriever weighing 28 kg was referred to the Veterinary Teaching Hospital of Azabu University for detailed examination of an oral mass and lameness. At the first admission, the activity and appetite were normal, although weight loss was seen (body condition score: 2). Physical examination revealed large (diameter: 3.5 cm) and small (diameter: 0.8 cm) soft round masses in the left maxilla (Fig. 1a), and another small mass (diameter: 0.8 cm) was located in the gums of the left mandible. The complete blood count and blood chemistries were normal

(Table 1a). Total protein, albumin, and A/G ratio were 6.4 g/dl, 3.1 g/dl, and 0.94, respectively. The protein concentration and specific gravity of urine were 0.273 g/dl and 1.040, respectively. No monoclonal gammopathy was seen on electrophoresis of serum and urine samples. X-ray examination revealed multiple sites of osteolysis (punched-out areas) in the jaw bone, thoracic vertebrae, lumbar vertebrae (Fig. 1c), breast bone, rib, ilium, and ulnar bone (Fig. 1d), but no other abnormality indicating the existence of tumors was seen. Eight days after the initial admission, biopsy samples were obtained from a spur of the ilium with osteolysis and the oral mass. On histopathological examination, the sample obtained from the spur showed normal bone and cartilaginous tissue. The specimen from the oral mass contained large myeloid-like cells with dark-blue cytoplasm and lobed nuclei (Fig. 2), but the presence of a plasmacytoma was not confirmed. The case was suspected to be a myeloid neoplasm based on the histopathological examination. Twenty-three days after the initial admission, bone marrow aspiration was performed. The bone marrow was normocellular, and the myeloid:erythroid ratio was 0.72. The percentage of blasts was normal (2.1%), and erythroid and myeloid cells were well differentiated (Table 1b). A small number, 5% of all nucleated cells, of large atypical mononuclear cells like the tumor cells derived from the oral mass were seen in the bone marrow. Cytochemical examinations, including peroxidase and non-specific esterase (α -butyrate) stains, were negative for the large atypical mononuclear cells seen in the oral mass and bone marrow tissue. Because myelocytic and monocytic neoplasms were excluded by the results of the cytochemical examination, clonal analysis of lymphocytes was performed. The polymerase chain reaction (PCR) for antigen receptor rearrange-

* CORRESPONDENCE TO: TSUCHIYA, R., Laboratory of Internal Medicine II, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagami-hara, Kanagawa 229-8501, Japan.
e-mail: tsutiyar@azabu-u.ac.jp

Table 1. The results of blood and bone marrow examination

a) Complete blood count and chemistry		(b) Myelogram	
WBC	4,250 / μ l	Rubriblast	0.6%
Band neutrophil	47 / μ l	Basophilic rubricyte	4.7%
Segmented neutrophil	2,035 / μ l	Polychromicrubricyte	14.2%
Lymphocyte	1,743 / μ l	Normochromic rubricyte	33.8%
Monocyte	340 / μ l		
Eosinophil	85 / μ l	Myeloblast	1.1%
RBC	5.41×10^6 / μ l	Promyelocyte	2.8%
HCT	35.7 %	Myelocyte	7.5%
PLT	25×10^4 / μ l	Metamyelocyte	4.9%
ALT	22.4 U/l	Band neutrophil	6.9%
ALP	95.7 U/l	Segmented neutrophil	15%
Glu	105 mg/dl		
TP	6.4 g/dl	Monoblast	0.4%
ALB	3.1 g/dl	Promonocyte	0.6%
BUN	8.6 mg/dl	Monocyte	0.2%
Cre	0.9 mg/dl		
T-CHO	256 mg/dl	Megakaryocyte	0.2%
TG	132 mg/dl		
T-bil	0.2 mg/dl	Atypical cell	5%
Ca	10.3 mg/dl		
IP	4.2 mg/dl	Blast in ANC	2.1%
Na	154 mmol/l	ME ratio	0.72
K	4.2 mmol/l		
Cl	112 mmol/l		
A/G	0.94		

ments (PARR) was used to clarify the neoplastic proliferation of lymphocytes and to determine the populations of B and T lymphocytes [1]. Clonal analysis was performed according to the protocol that was previously reported by Burnett *et al.* [1]. The results of clonal analysis were positive for the clonal expansion of B lymphocytes (IgH major) but not T lymphocytes in the oral mass tissues (Fig. 3). An extra-bone marrow plasmacytoma was excluded because there was no evidence of a mass of plasma cells was present in the oral cavity, skin, or gastrointestinal system on ocular, X-ray, ultrasound, and cytological examinations. Based on these results, the case was diagnosed as atypical B cell lymphoma.

The case was treated by multi-drug chemotherapy, including vincristine, L-asparaginase, cyclophosphamide, doxorubicin, and prednisolone. As induction therapy, vincristine (0.6 mg/m²) was administered intravenously (IV) and prednisolone (1 mg/kg, daily) was given orally on day 28. After administration of vincristine, facial paralysis was seen. When cyclophosphamide (50 mg/m², days 32, 33, and 34) was administered orally, the size of the oral masses decreased. After L-asparaginase (10,000 U/m²) was administered subcutaneously on day 38, severe vomiting and diarrhea occurred. Chemotherapy was discontinued, and the case was treated with an H₂ blocker and fluid therapy. Chemotherapy with vincristine (day 52) and cyclophosphamide (days 56, 57, and 58) was resumed by the referring veterinarian, but complete remission was not seen. To achieve complete remission, doxorubicin (1 mg/kg, IV on days 60, 82, 102, and 123) was administered by the referring veterinarian. By day 142, the size of the oral mass was reduced

and multiple osteolysis was improved. The diameter of the largest oral mass had decreased from 3.5 to 2.5 cm and other small masses had disappeared (Fig. 1b); fine needle biopsy confirmed the absence of neoplastic cells. Clinical signs of lameness, weight loss, and systemic osteolysis were considerably improved (Fig. 1e).

The occurrence of lymphoma in the oral mucosa is rare, and an atypical lymphoma similar to myeloid cells has not been reported in the veterinary field [13]. Furthermore, the morphology of the neoplastic cells was different from typical canine lymphoma; indeed, this is the first report describing an atypical lymphoma occurring in the oral region. Canine lymphoma may be histologically classified by the 2 most useful classification systems: the National Cancer Institute Working Formulation (NCI-WF) and the Kiel Classification [2, 8]. Both systems describe mitotic index, cell size, and cell shape. The NCI-WF system classifies lymphoma cells based on lymph node architectural pattern (diffuse vs. follicular) and cell type (small cleaved, large cell vs. immunoblastic) [2]. In addition to the descriptive pattern, the Kiel system also incorporates morphology (centroblastic, centrocytic, and immunoblastic) and immunophenotype [8]. However, the type of lymphoma cell with atypical myeloid cell-like nuclei and a large quantity of cytoplasm has not been defined. In human medicine, non-Hodgkin's lymphoma occurring in the infraorbital region has been reported; the morphology of the neoplastic cells was atypically large lymphocytes, and the immunophenotype was confirmed as B cell lymphoma [7]. We think that the present case is similar because the cell immunophenotype and anatomic location of the tumor corresponded.

Fig. 1. Oral mass and radiography on first admission and after chemotherapy. The oral mass at the time of the initial admission (before chemotherapy) was large and round and the tissue was soft. b: The oral mass after chemotherapy (142 days after admission) was smaller, and the tissue showed scar formation and hardening. c: Osteolysis (punched-out sites) of the lumbar vertebrae at admission; d: osteolysis of the left ulnoradial bone at admission. e: The osteolysis of the left ulnoradial bone was decreased after chemotherapy (142 days after admission).

Fig. 2. Cytological findings of oral mass biopsy (Wright-Giemsa stain, $\times 600$), tumor cells were large and round cytoplasm with round or lobed nuclei.

The present case also showed severe systemic osteolysis as a paraneoplastic syndrome, which was seen in multiple myeloma, lymphoma, osteosarcoma, hemangiosarcoma, and transitional cell carcinoma [4]. In general, osteolysis is due to secretion of parathyroid hormone-related protein (PTHrP) by lymphoid tumor cells; most cases were defined as T cell type lymphoma [6, 9]. In addition, systemic osteolysis with punched-out sites is often seen in multiple myeloma due to osteoclast-activating factor (OAF) produced by plasmacytes and mature B cells. Hematological malignancies such as myeloma and adult T-cell lymphoma/leukemia can induce bone lesions because many of the factors that increase osteoblast formation and activation are produced by lymphocytes and monocytes [11]. For example, lymphocytes can produce interleukin-1 (IL-1), IL-6, colony-stimulating factors, and lymphotoxin. Further, macrophages can produce tumor necrosis factor- α (TNF- α), IL-1, granulocyte-macrophage-colony stimulating factor (GM-CSF), IL-6, and macrophage-colony stimulating factor

Fig. 3. PCR analysis of immunoglobulin gene rearrangement in B lymphocytes. Clonal analysis of immunoglobulin heavy chain (IgH) by PCR was performed as previously described by Burnett *et al.* [1]. Briefly, DNA samples were prepared using the QIAamp DNA mini kit from Qiagen. For each sample, PCR was performed using CB1 and CB2 primers (IgH major) [1]. The PCR products were then electrophoresed on a 10% native polyacrylamide gel. Amplifications for IgH minor (a smaller population of IgH gene rearrangements), TCR γ (T-cell receptor γ gene rearrangements), and C μ (positive control for DNA extraction and PCR amplification) were also performed and analyzed (data not shown). Discrete bands (about 120 bp) show the presence of clonal expansion of B lymphocytes. Lane 1, oral mass; lane 2, positive control obtained from a case of confirmed canine lymphoma; lane 3, negative control using distilled water instead of template DNA.

(M-CSF). These cytokines have been shown to increase osteoblast formation and/or osteoclastic bone resorption. We suspect that the osteolysis might be caused by OAF as multiple myeloma in this case of B cell lymphoma. In fact, a human case presenting with multiple osteolysis due to a diffuse large B-cell lymphoma in which overexpression of an OAF-related gene, macrophage inflammatory protein-1 alpha (MIP-1 α), has been reported [10]. In the future, assessment of serum PTHrP and OAF concentrations will be required to clarify the pathogenesis of osteolysis related to atypical lymphoma.

In general, the detection of canine lymphoma relies on cytological or histological examination of lymphoid tissue and masses [2, 8]. However, it would be difficult to detect lymphoma in a cavity fluid that contains a large number of mature lymphocytes and in cases of low-grade lymphoma in the early stage of disease [8]. Burnett *et al.* proposed that molecular analysis of the rearrangement of the lymphocyte antigen receptor gene was the most useful assay for detecting the neoplastic proliferation of lymphoid tissue [1]. Immunoglobulin gene rearrangement in B cells and T cell receptor (TCR) gene rearrangement in T cells can be detected by the PCR method, which has been characterized as a highly sensitive and specific assay [1, 3]. In our case, the character of the tumor cells and the neoplastic proliferation could be defined by clonal analysis, but not by cytolog-

ical or histopathological examinations.

The dog was treated with multi-drug chemotherapy including vincristine, L-asparaginase, cyclophosphamide, doxorubicin, and prednisolone. We planned to use the COP protocol to treat the dog, but an adverse reaction was seen and remission was not achieved. Subsequently, treatment with doxorubicin reduced the mass and ameliorated the osteolysis. In general, cutaneous epitheliotropic lymphoma is known to be refractory to chemotherapy, so a therapeutic regime using a strong agent like doxorubicin may be needed to treat oral lymphomas like this case. Risbon *et al.* indicated that CCNU (Lomustine) treatment was effective for 83% of canine cutaneous epitheliotropic lymphoma cases, so this agent may be useful to achieve complete remission of oral lymphoma [12].

We conclude that this dog exhibited atypical lymphoma and that analysis by PCR was the most helpful tool to confirm this type of lymphoma.

REFERENCES

1. Burnett, R. C., Vernau, W., Modiano, J. F., Olver, C. S., Moore, P. F. and Avery, A. C. 2003. *Vet. Pathol.* **40**: 32–41.
2. Carter, R. F., Valli, V. E. and Lumsden, J. H. 1986. *Can. J. Vet. Res.* **50**: 154–164.
3. Dreitz, M. J., Ogilvie, G. and Sim, G. K. 1999. *Vet. Immunol. Immunopathol.* **69**: 113–119.
4. Dennis, R. 2003. pp. 41–60. *In: BSAVA Manual of Canine and Feline Oncology*, 2nd ed. (Dobson, J. M., Duncan, B., and Lascelles, X. eds.), BSAVA, Gloucester.
5. Ettinger, S. N. 2003. *Clin. Tech. Small. Anim. Pract.* **18**: 92–97.
6. Fournel-Fleury, C., Ponce, F., Felman, P., Blavier, A., Bonnefont, C., Chabanne, L., Marchal, T., Cadore, J. L., Goy-Thollot, I., Ledieu, D., Ghernati, I. and Magnol, J. P. 2002. *Vet. Pathol.* **39**: 92–109.
7. Gedik, R., Gedik, S., Goze, F. and Develioglu, H. 2003. *J. Am. Dent. Assoc.* **134**: 1353–1355.
8. Greenlee, P. G., Filippa, D. A., Quimby, F. W., Patnaik, A. K., Calvano, S. E., Matus, R. E., Kimmel, M., Hurvitz, A. I. and Lieberman, P. H. 1990. *Cancer* **66**: 480–490.
9. Kubota, A., Kano, R., Mizuno, T., Hisasue, M., Moore, P. F., Watari, T., Tsujimoto, H. and Hasegawa, A. 2002. *J. Vet. Med. Sci.* **64**: 835–837.
10. Matsushashi, Y., Tasaka, T., Uehara, E., Fujimoto, M., Fujita, M., Tamura, T., Honda, T., Kuwajima, M., Shimoura, Y., Mano, S., Nagai, M. and Ishida, T. 2004. *Leuk. Lymphoma* **45**: 397–400.
11. Risbon, R. E., de Lorimier, L. P., Skorupski, K., Burgess, K. E., Bergman, P. J., Carreras, J., Hahn, K., Leblanc, A., Turek, M., Impellizeri, J., Fred, R. 3rd., Wojcieszyn, J. W., Drobatz, K. and Clifford, C. A. 2006. *J. Vet. Intern. Med.* **20**: 1389–1397.
12. Roodman, G. D. 1997. *Cancer* **80**: 1557–1563.
13. Vail, D. 2003. pp. 276–292. *In: BSAVA Manual of Canine and Feline Oncology*, 2nd ed. (Dobson, J. M., Duncan, B. and Lascelles, X. eds.), BSAVA, Gloucester.
14. Vail, D. M., MacEwen, E. G. and Young, K. M. 2001. pp. 558–590. *In: Small Animal Clinical Oncology*, 3rd ed. (Withrow, S. J., and MacEwen, E. G. eds.), Saunders, New York.