Canine Multicentric Large B Cell Lymphoma with Increased Mott Cells Diagnosed by Flow Cytometry

Yeseul Yang*,**, Jae-Ha Jung***, Sung-Hyun Hwang*** and Yongbaek Kim*,*****

*Laboratory of Clinical Pathology, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea
**BK 21 FOUR Program for Future Veterinary Medicine Leading Education and Research Center, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea
***Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea

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Abstract: A 12-year-old dog was referred due to multiple superficial lymphadenopathy. On cytology, each lymph node showed different cell populations where some of them consisted of intermediate to large lymphocytes with frequent Mott cells. Presence of Mott cells along with immature lymphocytes made the cytological diagnosis challenging, and therefore, supplementary diagnostic tests including PCR for Antigen Receptor Rearrangement (PARR) assay and flow cytometry were performed. This case report illustrates the value of flow cytometry in the diagnosis of lymphadenopathy with ambiguous cytologic findings.

Key words: flow cytometry, multicentric B cell lymphoma, Mott cells, immunophenotype, dog.

Introduction

Lymphadenopathy is commonly associated with inflammation, immune stimulation or neoplasia. Fine needle aspiration (FNA) is a useful tool for the differential diagnosis of lymphadenopathy because each entity shows distinct composition of lymphoid and other cells (4). Reactive lymphoid hyperplasia consists of heterogeneous population with predominantly small lymphocytes and various number of plasma cells. If large lymphocytes of which nuclei are 1.5 to 3 times the size of the red blood cells are more than 50% of the cell population, the diagnosis of lymphoma is reliable, and more than 80% makes the diagnosis confirmatory (2). However, in case neoplastic cells do not increase enough to meet the diagnostic criteria, additional diagnostic tools such as polymerase reaction for antigen receptor rearrangement (PARR) or flow cytometry can be supportive.

Case Report

A 12-year old castrated male Shih-tzu dog was presented with enlargement of bilateral submandibular and axillary lymph nodes. At the time of initial admission, FNA from submandibular lymph node revealed heterogeneous population of lymphocytes with frequent Mott cells and was diagnosed as reactive lymphoid hyperplasia at local animal hospital

Fig 1. (a), (b) Cytology of submandibular lymph node at initial diagnosis. Arrows indicate Mott cells. Modified Wright’s stain, x40. Bar = 50 μm.
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(Fig 1). However, there was no evidence of underlying diseases. Moreover, prescapular and inguinal lymph nodes became enlarged despite administration of 1 mg/kg methylprednisolone for seven days. As the disease progressed, the patient was referred to the Veterinary Medicine Teaching Hospital, Seoul National University for the further evaluation. On physical examination, enlargement of multiple superficial lymph nodes was observed.

Complete blood counts (CBC) revealed mild anemia and lymphocytosis (Hct 34.1%, RI 37.1-57.0; Lymphocytes 6,965/μl, RI 1,000-4,000). Serum biochemistry tests showed increases of liver enzymes (ALT 143 U/L, RI 5.8-83.3; AST 47 U/L, RI 11.7-42.5; ALP 727 U/L, RI 0-97.9, respectively), which may be attributable to the treatment with steroids with no other abnormality. Thoracic X-ray radiography showed no remarkable findings, but abdominal ultrasonography revealed honeycomb sign in the spleen and enlargement of bilateral medial iliac lymph nodes (Fig 2).

FNA performed on the right submandibular, prescapular and inguinal lymph nodes along with spleen, and the smeared slides were prepared with modified Wright’s stain (Diff-quik stain) for microscopic examination. All the lymph nodes showed high cellularity of lymphoid cells but different composition (Fig 3). In submandibular and inguinal lymph nodes,

Fig 2. Abdominal ultrasonography. A: Honeycomb-like lesion of splenic parenchyma. B: The size of left medial iliac lymph node was 1.93 × 1.56 cm.

Fig 3. Fine-needle aspirates of lymph nodes and spleen. Modified Wright’s stain, (a) spleen, x40 (b) submandibular lymph node, x40 (c) prescapular lymph node, x 40 and (d) inguinal lymph node, x100. Both Mott cell (arrow) and large lymphocyte (arrowhead) are found. Bar = 50 μm.
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approximately 70% of the nucleated cells were intermediate to large lymphocytes. Two to three round cells with eccentric nucleus and spherical inclusions packed in cytoplasm, which were consistent with Mott cells, were seen per high power field (hpf). In prescapular lymph node, more than 60% of the nucleated cells were small lymphocytes with dense nuclei. Cytology of the spleen showed predominantly small lymphocytes, and increased number of megakaryocytes and erythroid precursor cells. These cytological findings led to a tentative diagnosis of large cell lymphoma in the multiple lymph nodes and extramedullary hematopoeis in the spleen. However, marked increase of Mott cells were observed in both submandibular and inguinal lymph nodes, which was not a typical finding to support the diagnosis of lymphoma. Moreover, the composition of lymphoid cells in prescapular lymph node was more consistent with reactive lymphoid hyperplasia. In order to resolve the diagnostic challenge, additional tests were carried out.

To determine clonality and immunophenotype of the lymphoid cells, PARR assay and flow cytometry were performed for the cells obtained by FNA from the inguinal lymph node. Four primer sets were used for the detection of rearranged TCR gene in T cells and two primers for rearranged IgH gene in B cells according to the published report (17,19). Primers for V segments (V\(\gamma_a\): 5’-CGTGTACTACTGGCT-GCCTGG-3’ and V\(\gamma_b\): 5’-GGCTGATTACTGTGCCTGCT-GTGG-3’) and primers for J segments (J\(\gamma_a\): 5’-TACCTTCTG (C/T)AAATATCTTG-3’ and J\(\gamma_b\): 5’-TGTGCGAGCC-AAGCATTTGTT-3’) were used to detect TCR genes. For detection of IgH gene in B cells, primers for V region and J region were designed as, 5’-ACACGGCC(A/C/G)TGATTACTGTG-3’ and 5’TGGAGAGCCTTGTGACC-3’, respectively. For normalization, a primer set within a single exon of the juxtamembrane domain sequence of the canine c-kit was designed: forward primer, 5’-CCCATGTAGAAGATCTAGTTAGAG-3’ and reverse primer, 5’-GTTCCTAAAGTCATTTGTAGACTG-3’. PARR assay on the cells aspirated from inguinal lymph node revealed monoclonality of B cell lineage (Fig 4). For immunotyping, cells isolated from inguinal lymph node were analyzed by a FACSverse (Becton Dickinson, Mountain view, CA, USA) using monoclonal primary antibodies including mouse anti – dog CD3 – FITC (AbD Serotec Cat# MCA1774F) and mouse anti – dog CD21 – Alexa Flour® 647 (AbD Serotec Cat# MCA1781A647).

**Fig 4.** PCR analysis of B cell and T cell receptor clonal rearrangement. Specific bands were seen in lane 8-10, indicating the possibility of B cell lymphoma. M: Marker; Lane 2-5: V\(\gamma_a\)-J\(\gamma_a\), V\(\gamma_a\)-J\(\gamma_b\), V\(\gamma_b\)-J\(\gamma_a\) and V\(\gamma_b\)-J\(\gamma_b\) primer set; Lane 7-9: IgH-J-IgHV 1-3 primer set; Lane 10: c-kit (positive control).

**Fig 5.** Flow cytometry of aspirated cells from inguinal lymph node (a) with or (b) without antibody stain. About 94.7 percent of lymphocytes were positive to CD21 antibody.
More than 90% of the cells were CD21 positive (Fig 5). The serum protein electrophoresis showed discrete band in the beta region which is consistent with a monoclonal gammopathy (Idexx Laboratories, INC). Immunoglobulin quantification was not performed. Based on the results of cytological and phenotypic analyses, the patient was diagnosed as multicentric large B cell lymphoma with increased Mott cell. The owner elected palliative therapy and management and therefore the patient was managed with medication for pain control only. The size of the superficial lymph nodes became larger and anorexia got worse. No abdominal scan was performed because the owner did not want further check-up. Three months later, the patient died at home and necropsy formed because the owner did not want further check-up. Three months later, the patient died at home and necropsy formed because the owner did not want further check-up.

**Discussion**

Lymphoma is the most common hematopoietic neoplasm in dogs. The clinical characteristics of the canine lymphoma is similar to that of the human, so the criteria for diagnosis and classification for human lymphoma have been applied for canine counterpart (13). It is generally diagnosed by FNA cytology and confirmed by proof of monoclonality. However, plasma cell differentiation has been reported in a few subtypes of lymphoma. Plasmablastic lymphoma and lymphoplasmacytic lymphoma are rare types of lymphoma, which are presented as generalized lymphadenopathy (10). Because the neoplastic lymphocytes have plasmacytic morphology, the microscopic features of these tumors are similar to plasmacytoma but the difference is that they arise in lymph nodes. They are also classified as plasmablastic variant of diffuse large B cell lymphoma. In human, a subtype of the lymphoma called Hodgkin lymphoma is composed of heterogeneous cell population including small lymphocytes, plasma cells, and eosinophils with specific cell called Reed-Sternberg cell (12) that was not detected in this case.

Mott cell is characterized as oval shape with eccentric nuclei and cytoplasm filled with vacuolar granules called Russel bodies (2). These granules are the trapped immunoglobulins produced in response to antigen stimulation. Since the plasma cells are differentiated B cells, neoplastic lymph node with Mott cell indicates the lymphoma of B cell origin (11). However, the systemic antigen stimulation can affect the lymph node even in lymphoma patients, and increase of plasma cells are differentiated B cells, neoplastic lymphocytes. Although the lymph node in this case had increased Mott cells, still the predominant cells were large lymphocytes.

Each subtype of lymphocytes has distinct expression pattern of cell surface protein (18). Therefore, cluster of differentiation (CD) molecules are used as common marker for cell lineage and flow cytometry has been widely used for detecting such molecules. The common surface markers include CD45 for leukocytes, CD21 for B cells, and CD3 for T cells (1). Flow cytometry allows to determine whether the population is homogenous or not, but also can detect the aberrant pattern (6). The immunophenotype of lymphoid cells in the present case was consistent with B cell lymphoma and the Mott cells were possibly differentiated from the neoplastic lymphocytes.

Lymphoma is the clonal expansion of lymphocytes and the confirmation of molecular clonality is supportive of lymphoma diagnosis. Because each lymphocyte has unique sequences in their antigen receptor genes, PARR has been commonly used for the detection of monoclonality in lymphoproliferative diseases (9). In lymphoid malignancies, the neoplastic lymphocytes express the same DNA sequence that is a target for clonality assessment. The PCR results in the present case indicated that the neoplastic cells were B cell in origin. All three lanes for IgH VJ region had two discrete bands with same size. Bi-clonal rearrangement is not uncommon feature of canine lymphoma (3). However, one of the bands might represent the gene rearrangement of Mott cells, as shown in a published report that the neoplastic plasma cells were monoclonal (7). Alternatively, since the somatic mutation occurs during the plasma cell differentiation from B cell, it is possible that neoplastic lymphocytes and Mott cells in the present case have different gene sequences. Single cell analysis is warranted to identify the origin for each band.

Several cases of B cell lymphoma with Mott cell differentiation are reported in veterinary medicine (5,8,14-16). The percentages of the Mott cells were variable between the cases, but all of the cases were confirmed as B cell lymphoma with monoclonality by PARR. However, this is the unique case that cytopathologic evaluation was applied in both early and late stages, showing the progression of the lymphoma.

**Conclusions**

Cytological diagnosis of lymphadenopathy could be challenging, particularly for the sample that is composed of heterogeneous lymphoid cells with increased plasma cells and Mott cells. The present case illustrates the potential value of supplemental diagnostic tools such as PARR and flow cytometry for the diagnosis of canine lymphoma.

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**Conflict of Interest**

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**References**