Comparison of polymerase chain reaction for antigen receptor gene rearrangement and flow cytometric analysis for the diagnosis of canine lymphoma

Ru-Hui Song, Do-Hyeon Yu¹, Jun-Hwan Kim, Hyun-Seok Lee, Da-Mi Lee, Chul Park, Il-Jung Yu, Jin-Ho Park*

College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea
¹College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, USA

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Abstract

Lymphoma is the most common hematopoietic malignancy in dogs. Diagnosis of lymphoma is classically performed by morphological assessment and immunohistochemistry. But some cases in the early stage are difficult to distinguish and need more objective and accurate methods. So, Polymerase chain reaction (PCR) for antigen receptor rearrangements (PARR) and flow cytometric immunophenotype of lymphoma have been developed continuously. In this study, we performed these two methods to classify lymphoma type in 3 cases. According to PARR analysis, B cell origin lymphoma was diagnosed in two of three cases by testing PBMC and lymph node. All fine needle aspiration (FNA) samples of lymph nodes had high expression of CD21 on >88% of total cell population and PBMC samples also showed high expression of CD21 on >30% of total lymphocytes in those two cases, while the expression of CD3, CD4 and CD8 was absent. These results suggest that concurrent use of PARR and flow cytometric immunophenotype is more effective and valuable tool for the diagnosis and monitoring of canine lymphoma patients.

Key words : Flow cytometry, PARR (PCR for antigen receptor rearrangements), Lymphoma

INTRODUCTION

Lymphoma is the most common hematopoietic malignancy in dogs and the annual incidence is estimated to be 24 cases in 100,000 dogs (Dorn et al, 1967). Canine lymphoma typically is a diffuse neoplasm resulting in destruction of the lymph node architecture by a monomorphic population of neoplastic lymphocytes (Bartolo and Viswanatha, 2001). This characteristic makes possible to diagnose many canine lymphomas by only cytological test without knowledge of node architecture (Gibson et al, 2004).

So, diagnosis of lymphoma is easily performed by morphological assessment and immunohistochemistry. But other methods like Polymerase chain reaction (PCR) for antigen receptor gene rearrangements (PARR) and flow cytometric immunophenotype are needed. For example, in the case of any suspect B-cell proliferations when morphology and immunophenotyping are not conclusive; all suspect T-cell proliferations; lymphoproliferations in immunodeficient patients, including post-transplant patients; evaluation of the clonal relationship between two lymphoid malignancies in one patient or discrimination between a relapse and a second malignancy; further classification of a malignancy via Ig/TCR gene rearrangement patterns or particular chromosome aberrations; staging of lymphomas; the early stage of disease; difficult to distinguish, flow cytometric immunophenotype and PARR should be performed (Culmsee et al, 2001; Culmsee and Nolte, 2002; van Dongen et al, 2003).
In veterinary medicine, more researches about these two methods for canine lymphoma are needed. So we performed these two methods to classify lymphoma type in 3 cases.

**MATERIALS AND METHODS**

**Animals**

We examined one castrated male dog and two female dogs. Two of them were admitted to Animal Medical Center of Chonbuk National University and one was referred to Haemaru Animal hospital. Samples of three dogs were requested for PCR amplification test and FACS analysis at our laboratory.

All the dogs presented generalized enlarged lymph nodes, anorexia, depression and abdominal distention. They were diagnosed as multicentric lymphoma based on cytological examination. Case 1 was a seven-year-old neutered male Shih-Tzu was presented for anorexia, lethargy and generalized enlarged lymph nodes. It was stage 4b of WHO criteria on cytological examination. Case 2, a five-year-old female schnauzer, was admitted with the history of generalized enlarged lymph nodes, depression, inappetence, tachypnea and abdominal distention. Clinico-pathologic revealed mild leukopenia, anemia and slightly increased alkaline phosphatase (ALP 508 U/L). In diagnostic imaging, splenomegaly and enlarged sublumbar lymph node were shown. And high-grade lymphoma was diagnosed. The size of more than 80% of total lymphocytes was medium to large. Case 3, an unknown aged female Shih-Tzu, was presented for ascites, icterus, generalized enlarged lymph nodes, edema and melena. This case was stage 5b.

**Sample collection**

FNA samples of the neoplastic lymph nodes were obtained and used for cytological test, flow cytometric analysis and PARR. For flow cytometric immuno-phenotype, the samples were diluted in PBS and remaining cells were immediately stored at −20°C until used.

For isolation of Canine PBMC, blood was collected into 6 ml potassium EDTA treated tubes. Peripheral blood mononuclear cells (PBMC) were immediately isolated by centrifugation at 700×g for 30 min through Histopaque® 1,119 and 1,077 (Sigma-Aldrich, St. Louis, Mo, U.S.A.) The PBMC layer was harvested. 5 ml of lysis buffer (83% ammonium chloride solution, pH 7.2) was added to remove the residual RBC and incubated for 5 min and then, centrifuged at 200×g for 10 min. PBMC that removed residual RBC were washed twice with PBS by centrifugation at 200×g for 10min. Cell counts were assessed by VET ABC impedance cell counter. Cell purity was determined by a conventional diff-quick method.

**Detection of the rearrangements of antigen receptor genes by using PCR**

Genomic DNA was extracted from FNA samples and PBMCs of patients using the QIAamp Blood mini kit (Qiagen). 100 ng of genomic DNA was amplified using 4 sets of primer for V and J regions. Amplification of immunoglobulin and T-cell receptor gamma sequences was performed using previously described primers (Burnett et al, 2003).

Primers used for the amplification of Ig major and minor CDR3 and TCRγ CDR3 sequences are, CB1/CB2 (5’-CAG CCT GAG AGC CGA GGA CAC-3’/5’-TGA GGA GAC GGT GAC CAG GGT-3’), CB1/CB3 (5’-CAG CCT GAG AGC CGA GGA CAC-3’/5’-TGA GGA CAC AAA GAG TGA GG-3’) and TCRγ1/ TCRγ2/ TCRγ3 (5’-ACC CTG AGA ATT GTG CCA GG-3’/5’TCT GTT GGG A/GTG A/GTG TAC/T TAC TAC TGT GCT GTC TGG-3’), respectively.

To ensure the DNA could be amplified, positive control primers were used to amplify the constant region gene of IgM (Cμ). The primers of Cμ are Sigm1/Sμ3 (5’-TTC CCC CTC ATC ACC TAC TGT GA-3’/5’-GGT TGT TGA TTG CAC TGA GG-3’). After PCR amplification, the PCR products were analyzed by 12.5% native polyacrylamide gel electrophoresis.

**Flow cytometry**

Flow cytometry was performed with the BD flow