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Clinical Usefulness of Peripheral Blood Lymphocyte Subsets in Canine Lymphoma

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INTRODUCTION

The classification of canine lymphoid malignancies into groups based on cell of origin (B- or T-cell types) is possible with immunophenotypic analysis. A high frequency of B-cell lymphomas has been described; about 30% of lymphomas are of T-cell origin (Teske *et al*., 1994a; Fournel-Fleury *et al*., 1997; Grindem *et al*., 1998). In human medicine the major diagnostic role of blood lymphocyte subset typing is the screening for non-Hodgkin's lymphomas with peripheral blood involvement and their differentation from reactive lymphoproliferative conditions (Thalhammer-Scherrer *et al*., 2000). The aim of this project is to evaluate the diagnostic efficacy of blood immunophenotyping in dogs with lymphoma in order to detect the blood involvement and the prognostic benefits.

MATERIALS AND METHODS

Forty-eight dogs, 32 males and 16 females, ranging from 3 to 14 years old, of varying breeds, were examined. The diagnosis of lymphoma was performed on cytological smears. The immunophenotype was detected from lymph node cellular suspensions or cavity fluids using flow cytometry (FACScan Becton Dickinson): 36 cases were $CD21^+$ B-cell, 7 were $CD4^+$ T-cell, 3 were $CD8^+$ T-cell and 2 were $CD4^-CD8^-$ T-cell lymphomas. A complete blood count and a phenotypic analysis of blood lymphocyte subpopulations were performed. The following monoclonal antibodies, specific for canine leukocyte antigens, were used: CD21 (CA2.1D6), IgG $F(ab')_2$ and IgM, CD5 (DH3B), CD3 (CA17.2A12), CD4 (CA13.1E4), CD8 (CA9.JD3), CD45RA (CA4.1D3), CD49d (CA4.5B3) and CD45 (CA12.10C12). Except for CD5 (VMRD, Pulmann, Washington, USA) and IgG, IgM (Jackson Immunoresearch, West Grove, PA, USA), all monoclonal antibodies were kindly provided by Dr P.F. Moore (U.C.

Davies, CA, USA). The results were compared to reference values of a control goup of 50 healthy dogs (Guglielmino *et al*., 1995).

RESULTS

In agreement with Jain (1993), the complete blood count showed a mild to modest anaemia in 46% of the dogs, thrombocytopenia in 44% and mild (18,300/μl) to extreme (342,000/μl) leukocytosis in 33%. Only 19% of cases exhibited lymphocytosis; lymphoblasts were present for 12 dogs (25%), and 15% were without lymphocytosis. In 10% of cases leukopenia was observed and lymphopenia was observed in 25% of cases. Five out of 36 (14%) B-cell lymphomas showed $CD21^+$ B-cell lymphocytosis (ranging from 5520 to 115,670/μl); four cases showed a moderate increase of B lymphocytes without lymphocytosis. A $CD8^+$ T-cell increase was observed in seven B-cell lymphomas. This is also noted for human patients with neoplastic disorders, where an increase in the so-called 'anti-tumour' cytolytic $CD8^+$ T lymphocytes occurs (Quaglino *et al*., 1993). Two out of seven $CD4^+$ T-cell lymphomas showed lymphocytosis (17,680 and 45,050/μl) with an increase in $CD4^+$ T lymphocytes. Five dogs (71%) exhibited an increased CD4/CD8 ratio: in three cases this was due to an absolute increase in $CD4^+$ T cells. One dog showed an abnormal immunophenotype ($TCD4^+CD45^-$) both in the lymph node and in the peripheral blood. In two out of the three dogs with $CD8^+$ T-cell lymphoma a mild increase in the absolute count of $CD8^+$ T lymphocytes, without lymphocytosis, was noted; one of these showed the same abnormal immunophenotype as the lymph node ($TCD8^+CD45^-$). Extreme lymphocytosis (12,690 and 338,580/μl), due to neoplastic cells, was observed for two dogs with $CD4^-CD8^-$ T-cell lymphoma.

DISCUSSION

In dogs with lymphoma the total and differential leukocyte counts and the presence of atypical lymphocytes were very variable: indeed blood involvement was not always detectable on a simple complete blood count. The detection of neoplastic cells (5 T-cell and 4 B-cell lymphomas) with the same immunophenotype as the origin tumours, also without lymphocytosis and cells with atypical morphology, was possible using flow cytometric analysis of peripheral blood. The immunophenotype of the peripheral blood was able to provide more precise information about systemic involvement. In 75% (9/12) of T-cell lymphomas and in 25% (9/36) of B-cell lymphomas a systemic involvement was present, confirming a poorer prognosis for T-cell lymphomas as compared with B-cell lymphomas (Teske *et al*., 1994b). Simultaneous immunophenotyping of blood, lymph node and bone marrow from dogs with lymphoma could represent an important diagnostic and prognostic tool. Our study supports the potential use of blood immunophenotyping as a sensitive tool in the

detection of leukaemic cells, facilitation of tumour staging and monitoring of remission or relapse.

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