The Immunophenotype and Hematology of Canine Leukemia

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**Chronic Lymphocytic Leukemia (CLL)**

Canine CLL differs markedly from human CLL. In the largest canine study to date, canine chronic lymphocytic leukemia (CLL) occurred in older dogs (mean age 9.75 years; range 1.5 - 15 years; n=73 cases). Blood lymphocyte counts ranged from 15,000/ul to 1,600,000/ul. Surprisingly, 73% of CLL cases involved proliferation of T lymphocytes (CD3+), and 54% of CLL cases had granular lymphocyte (GL) morphology. GL CLL’s were almost exclusively proliferations of T cells that expressed CD8 and the leukointegrin CD11d and more frequently expressed T cell receptor (TCR)αβ (69%) than TCRγδ (31%). Canine GL CLL followed a typically indolent clinical course. The non-GL T cell CLL cases (19% of CLL) involved proliferation of TCRαβ T cells in which no consistent pattern of CD4 or CD8 expression was found. B cell CLL, based on expression of CD21 (and lack of T cell antigens) or CD79a, accounted for only 26% of canine CLL cases. No cases of canine B cell CLL expressed CD5. CD1c expression was present in 95% of canine B cell CLL. These results are in marked contrast to people where greater than 95% of CLL’s involve proliferation of CD5+ B lymphocytes.

Anemia (< 0.37 L/L) is a common finding (58% of cases) in canine CLL. Of the cases with anemia, 58% were classified as mild (Hct 0.30-0.36, reference interval 0.37-0.55 L/L), 34% as moderate (Hct 0.20-0.30 L/L) and 8% as severe (Hct <0.20 L/L). Almost all anemias were poorly responsive. Thrombocytopenia was present in 27% of cases and, in the majority of these cases (72%), it was relatively mild (120-200 x 10^9/L, reference interval 180-500 x 10^9/L). Absolute neutropenia (<3 x 10^9/L) was not observed in any case.

Canine B cell CLL appears to be a primary bone marrow disease. However, bone marrow involvement in T cell CLL involving proliferation of GL’s appears to occur relatively late in the course of the disease. The neoplastic expansion in these instances appears to originate in the spleen. In several cases of GL CLL, concurrent aspirates of spleen and marrow were available for assessment. In most of these cases, there was marked splenic infiltration with GL’s but bone marrow involvement was either minimal or inapparent. Occasionally, there was significant marrow infiltration but this was accompanied by a greater degree of splenic involvement. Interestingly, CD11d expression is relatively constrained in tissue; the splenic red pulp is the dominant site of CD11d expression in the hematopoietic system in both dogs and cats.

The different immunophenotypes observed in canine CLL do not appear to be associated with different clinical courses or confer different prognoses although long term follow up was not available for many of the cases in the above mentioned study. The disease runs a somewhat variable course, depending on how advanced it is at the time of initial diagnosis. Several very advanced cases with lymphocyte counts in excess of 500,000/ul (500 x 10^9/L), severe anemia and significant clinical signs achieved complete remissions with normalization of the lymphocyte counts. Many of the dogs were still alive, more than three years after the initial diagnosis, and this long term survival appeared to be independent of the different immunophenotypes present within this group. The immunophenotype of a given case did not change significantly over time or with disease progression in those instances where repeat immunophenotyping was performed. As with other types of canine CLL, canine GL CLL appears to respond favourably to standard prednisone and chlorambucil therapy.

**Acute Leukemia.**

Similar to the experience in people, immunophenotyping also appears to be useful in the assessment of acute leukemias in dogs. In the aforementioned study of canine CLL, a total of 38 cases of acute leukemia were also
Many cases of acute leukemia in the dog involve proliferation of cells with a primitive or immature morphology that is unhelpful in predicting possible lineage. Additionally, there appears to be a relatively poor correlation between morphologic appearance of the neoplastic cells and immunophenotype, confirming the necessity of immunophenotyping to determine the origin or lineage of the neoplastic cells. Similar to the situation in people, routine morphologic assessment appears to have significant limitations in the diagnosis of canine acute leukemia.

While lack of lymphoid antigen expression and expression of a constellation of myeloid associated antigens is very supportive of a myeloid origin, definitive immunophenotypic confirmation of AML in the dog is not currently possible. In an effort to rectify this situation, we have recently developed monoclonal antibodies specific to canine myeloperoxidase (Vernau W., Graham P. and Moore P.F., unpublished). Myeloperoxidase is considered to be the definitive myeloid specific marker. We have assessed 34 cases of acute leukemia with these canine specific anti-MPO antibodies (unpublished). Results thus far indicate that these canine specific MPO Mab are useful for the definitive diagnosis of AML in the dog. Thirteen of seventeen (13/17) canine acute leukemias were classified as AML on the basis of MPO positivity that confirmed their myeloid origin. The four MPO negative canine acute leukemias that were still classified as AML’s consisted of 2 Acute Megakaryoblastic Leukemias and 2 probable Acute Monoblastic Leukemias (although most of these were MPO positive). The Acute Megakaryoblastic Leukemias were confirmed on the basis of CD41 (GpIIb) positivity.

REFERENCES


We have subsequently assessed CD34 expression in greater numbers and a wider variety of canine hematopoietic neoplasia. We have found lack of CD34 expression in lymphoma (0/299, including 204 B cell lymphomas and 95 T cell lymphomas) and frequent CD34 expression in acute leukemias (10/10 B-ALL, 2/3 T-ALL, 9/10 AUL and 18/20 AML). Assessment of CD34 expression is useful for the differentiation of ALL (CD34+) from some cases of primary lymphoma with marked secondary leukemia (CD34-) and some unusual cases of CLL (also CD34-).

Most cases of acute leukemia (including GL acute leukemia) are fulminant diseases that are rapidly fatal. There appears to be no sex predisposition with the female: male ratio approximating 1.0. Common presenting signs are relatively non-specific and include hyporexia or anorexia, severe depression and lethargy, pyrexia and occasionally shifting leg lameness and bone pain. Lymphadenopathy is uncommon and, if present, is usually relatively mild. Weight loss is also uncommon.

Anemia is a frequent finding in non-GL acute leukemia (93% of cases), usually (88%) moderate to severe (mean Hct 0.25 L/L, range 0.10-0.41, reference interval 0.37-0.55 L/L) and non responsive. Sixty five percent of dogs with non-GL acute leukemia were neutropenic (<3000/ul or 3 x 10^9/L), and, in many of these cases (67%), the neutropenia was severe (<1000/ul or 1 x 10^9/L). Thrombocytopenia is also common (92% of non LGL acute leukemias) and often marked (<50,000/ul or 50 x 10^9/L). The frequency and severity of the anemia and thrombocytopenia observed in cases of GL acute leukemia is very similar to that seen in non-GL acute leukemias. In contrast, none of the dogs with GL acute leukemia were neutropenic. Most dogs (86%) with GL acute leukemia have neutrophilia (mean=34.5x10^9/L, range=8.4-102x10^9/L, reference interval 3-11.5x10^9/L) and this finding may assist in the diagnosis of GL acute leukemia versus AML.

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