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Comparison of serum cytokine levels between dogs with multicentric lymphoma and healthy dogs☆

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ABSTRACT

In humans, multiple cytokines have been linked to the development of lymphoma, and are relevant biomarkers for response to chemotherapy and prognosis. In contrast, only a few circulating cytokines have been studied in dogs with lymphoma. We prospectively enrolled thirty-one dogs newly diagnosed with multicentric lymphoma. Immunophenotype was determined by flow cytometry in all dogs, separating them into 2 subgroups: B cell lymphoma (n = 21) and T cell lymphoma (n = 10). Nineteen healthy dogs were enrolled in the control group. Circulating cytokine concentrations were measured using a commercial canine multiplex magnetic bead-based assay which included Interleukin-2 (IL-2), IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), Tumor Necrosis Factor-α (TNF-α), Interferon γ (IFN-γ), IFN-γ induced Protein-10 (IP-10), Keratinocyte Chemoattractant-like (KC-like), and Monocyte Chemoattractant Protein-1 (MCP-1). The serum levels of each cytokine were first compared between the lymphoma and control groups, and then between the B cell lymphoma, T cell lymphoma, and control groups. There was no significant difference between the lymphoma and healthy control groups regarding sex, age and weight. MCP-1, IL-6, and IL-10 were significantly higher in dogs with lymphoma compared to healthy dogs ($p < 0.01$, $p = 0.01$ and $p = 0.03$, respectively). MCP-1 and IL-10 were significantly higher in the B cell lymphoma group than in the healthy group ($p = 0.01$, $p = 0.01$, respectively). MCP-1 and IL-6 levels were significantly higher in the T cell lymphoma group than in the healthy group ($p = 0.02$, $p < 0.01$, respectively). IL-6 was significantly higher in the T cell lymphoma group than in the B cell lymphoma group ($p = 0.03$). Significant differences among the groups were found for IL-15 and KC-like, but they were affected by age and/or sex. There were no significant differences in serum IL-2, IL-7, IL-8, IL-18, GM-CSF, TNF-α, IFN-γ, and IP-10 between any of the groups. Significant differences in red blood cell, white blood cell, neutrophil, lymphocyte and monocyte counts were also found between the different groups of dogs. Our data showed different serum cytokine and peripheral blood cell profiles between dogs with lymphoma and healthy dogs, and between dogs with B cell and T cell lymphoma. Further study is necessary to investigate the role of these cytokines in lymphoma pathogenesis, response to treatment, and prognosis, and the influence of age, sex and blood cell counts on their expression.

1. Introduction

Lymphoma is one of the most common cancers in dogs, comprising 7 to 24% of all canine neoplasia (Withrow et al., 2013). Several clinicopathologic factors have proven useful to refine the prognosis, with immunophenotype being one of the most important: T cell lymphoma is associated with reduced survival compared to B cell lymphoma (Ruslander et al., 1997; Teske et al., 1994; Wilkerson et al., 2005). Approximately 60–80% of canine lymphomas are of B cell origin, and 10–38% of T cell origin (Fournel-Fleury et al., 2002; Greenlee et al., 1990; Ponce et al., 2010; Ruslander et al., 1997; Wilkerson et al., 2005). However, much remains to be learned about

Abbreviations: DLBCL, diffuse large B cell lymphoma; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon γ; IL, interleukin; IP-10, IFN-γ induced protein-10; KC-like, keratinocyte chemoattractant-like; MCP-1, monocyte chemoattractant protein-1; NHL, non-Hodgkin's lymphoma; RBC, red blood cell; TNF-α, tumor necrosis factor-α; WBC, white blood cell.

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lymphoma pathogenesis and the interactions with its microenvironment. In humans, chronic stimulation of the immune system is a risk factor for development of non-Hodgkin's lymphoma (NHL) (Vendrame and Martinez-Maza, 2011). People infected by Human Immunodeficiency Virus or Epstein Barr Virus are at greater risk to develop lymphoma, and this has been linked with changes in the host immune/cytokine environment (Epeldegui et al., 2006). Similarly, studies in immunocompetent people indicated a significant association between dysregulation of several cytokines and development of diffuse large B cell lymphoma (DLBCL), which is the most common lymphoma subtype in dogs (Edlefsen et al., 2014; Gu et al., 2010; Hussain et al., 2010; Saberi Hosnijeh et al., 2010). Cytokines can be produced by both neoplastic lymphocytes and the tumor microenvironment, including reactive lymphocytes, dendritic cells and macrophages. Therefore, evaluation of the circulating cytokine profile can reflect the activity of both the tumor and the host response (Kimby, 2015). Studies in NHL patients have demonstrated significantly increased circulating vascular endothelial growth factor (VEGF), interleukin-1 receptor antagonist (IL-1RA), soluble interleukin-2 receptor (sIL-2R), IL-6, IL-8, Chemokine Ligand 9 (CXCL9), IL-10, IP-10, IFN-γ, IL-12p40, IL-13, neopterin, Hepatocyte Growth Factor (HGF), and Macrophage inflammatory protein-1α/β (Mip-1α/β) compared to healthy patients; whereas IL-2 is higher in the subgroup of indolent NHL and IL-6 is higher in T lymphocyte-NHL patients than in B lymphocyte-NHL patients (Charbonneau et al., 2012; Fabre-Guillevin et al., 2006; Passam et al., 2008; Stasi et al., 1995; Wang et al., 2011). Moreover, some cytokines were shown to be valuable biomarkers for prognosis and response to therapy. For example, IL-6 and VEGF are independent predictors of response to therapy and survival in patients with NHL, while sIL-2R and IL-6 serum levels correlate with complete response, disease-free and overall survival (Fabre-Guillevin et al., 2006; Niitsu et al., 2002; Pedersen et al., 2005; Uskudar Teke et al., 2015). Circulating high IL-8, low IL-10, or low IL-16 before treatment are associated with complete response to treatment (Passam et al., 2008; Wang et al., 2011). Early changes in IL-6 and VEGF during the first three weeks of a CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin® [vincristine], Prednisone) chemotherapy protocol were independent predictors of response to therapy, and could prove useful as surrogate markers for this purpose (Pedersen et al., 2005).

There is a paucity of information in the veterinary literature regarding cytokines or other blood circulating biomarkers for canine lymphoma. In previous studies, dogs with lymphoma have been shown to have higher circulating MCP-1, activated-Matrix Metalloproteinase 9 (act-MMP9), VEGF, C-Reactive Protein (CRP) and haptoglobin, and lower Transforming Growth Factor- β (TGF-β) than healthy dogs (Aresu et al., 2014; Merlo et al., 2007; Mischke et al., 2007; Perry et al., 2011). CRP is useful in determining complete remission status after chemotherapy treatment, but failed as a marker of relapse (Merlo et al., 2007; Mischke et al., 2007). Act-MMP9 and VEGF are higher in T cell lymphomas than in B cell lymphomas, and in stage V compared to stages III–IV (Aresu et al., 2014). Elevated baseline serum MCP-1 is associated with lower disease-free interval in dogs treated with a CHOP protocol (Perry et al., 2011).

The primary objective of this study was to compare the serum levels of cytokines in dogs with multicentric lymphoma to healthy dogs. We hypothesized there would be a significant difference in cytokine levels between dogs with lymphoma and healthy dogs, and between dogs with B cell versus T cell lymphoma. As a secondary objective to try to better understand the origin of these cytokine differences, blood cell counts (red blood cells (RBC), white blood cells (WBC), neutrophils, lymphocytes, monocytes and platelets) were compared between the groups.

2. Materials and methods

2.1. Study groups

Dogs were prospectively enrolled into two groups between August 2014 and October 2015 at the Ontario Veterinary College Health Sciences Centre, University of Guelph. The first group consisted of dogs cytologically or histopathologically diagnosed with multicentric lymphoma (lymphoma group). Exclusion criteria included previous chemotherapy treatment (including L-Asparaginase); previous administration of systemic corticosteroids, nonsteroidal anti-inflammatory drugs (NSAID), or other immunomodulatory medications within the previous two weeks; systemic inflammatory diseases, and vaccination within the previous four weeks. To determine the immunophenotype of the lymphoma (B cell versus T cell), a fine-needle aspirate of a neoplastic peripheral lymph node was submitted for flow cytometry using a BD Accuri C6 flow cytometer.

The second group consisted of healthy adult dogs (control group) from the University of Guelph community. Similar exclusion criteria included: systemic inflammatory disease, systemic immunomodulatory medication within the previous two weeks (including corticosteroids and NSAID), and vaccination within the previous four weeks. For both groups, each dog had a complete physical examination and complete blood count (CBC) at the time of sampling, and biochemistry profile within the previous 2 weeks. For each control dog, a follow-up, either by telephone or email, was made 6 months later to ensure continued good overall health (i.e. no inflammatory or neoplastic disease).

This study was conducted in accordance with the guidelines of the Canadian Council on Animal Care, the requirements of the Animals for Research Act Revised Statutes of Ontario, and the institutional Animal Care Policy. This study was approved by the institution's Animal Care Committee. Written owner consent was obtained before study enrollment.

2.2. Sample collection and storage

For each dog, at least 2 mL of blood was drawn by jugular venipuncture into a dry syringe, and transferred into plastic Vacutainer tubes with no additive.[1] The blood was left at room temperature for at least 30 min until clotted, and then centrifuged at 1000×*g* for 10 min. Within 2 h of blood collection, serum was removed, aliquoted and stored at −80 °C for batch analysis of cytokine and chemokine concentrations.

2.3. Multiplex cytokine immunoassay kit

A commercial, canine-specific, antibody-coated magnetic microsphere-based multiplex cytokine immunoassay kit was used to simultaneously quantify 13 cytokines in each sample.[2] The following cytokines were measured: IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, TNF-α, IFN-γ, GM-CSF, KC-like (CXCL1), MCP-1 (CCL2), IP-10 (CXCL10). Multiplex immunoassays have proven to be sensitive, specific, rapid, require small sample volumes, and show a broad analytical and dynamic range (Keustermans et al., 2013). This particular kit has been successfully used in dogs with histiocytic sarcoma and mammary carcinoma, as well as several non-neoplastic diseases (Estrela-Lima et al., 2013; Floras et al.,

[1] BD Vacutainer plus plastic serum tubes; Becton Dickinson and Company, Franklin Lakes, NJ, USA.

[2] CCYTMG-90K-PX13 Canine Cyto/Chemo MAG Premix 13 Plex kit, EMD Millipore Corporation, Billerica, MA, USA.