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Research paper

Increased levels of interleukins 8 and 10 as findings of canine inflammatory mammary cancer

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ABSTRACT

Inflammatory mammary cancer (IMC) is a distinct form of mammary cancer that affects dogs and women [in humans, IMC is known as inflammatory breast cancer (IBC)], and is characterized by a sudden onset and an aggressive clinical course. Spontaneous canine IMC shares epidemiologic, histopathological and clinical characteristics with the disease in humans and has been proposed as the best spontaneous animal model for studying IBC, although several aspects remain unstudied. Interleukins (ILs) play an important role in cancer as potential modulators of angiogenesis, leukocyte infiltration and tumor growth. The aims of the present study were to assess serum and tumor levels of several ILs (IL-1α, IL-1β, IL-6, IL-8 and IL-10) by enzyme-immunoassay in dogs bearing benign and malignant mammary tumors, including dogs with IMC, for a better understanding of this disease. Forty-eight dogs were prospectively included. Animals consisted of 7 healthy Beagles used as donors for normal mammary glands (NMG) and serum controls (SCs), 10 dogs with hyperplasias and benign mammary tumors (HBMT), 24 with non-inflammatory malignant mammary tumors (non-IMC MMT) and 7 dogs with clinical and pathological IMC. IL-8 (serum) and IL-10 (serum and tissue homogenate) levels were higher in the dogs with IMC compared with the non-IMC MMT group. ILs were increased with tumor malignancy as follows: in tumor homogenates IL-6 levels were higher in malignant tumors (IMC and non-IMC MMT) versus HBMT and versus NMG and tumor IL-8 was increased in malignant tumors versus NMG; in serum, IL-1α and IL-8 levels were higher in the malignant groups respect to HBMT and SCs; interestingly, IL-10 was elevated only in the serum of IMC animals. To the best of our knowledge, this is the first report that analyzes ILs in IMC and IL-10 in canine mammary tumors. Our results indicate a role for IL-6, IL-8 and IL-10 in canine mammary malignancy and specific differences in ILs content in IMC versus non-IMC MMT that could have future diagnostic and therapeutic implications, to be confirmed in a larger series of IMC cases. These results help to support the validity of the IMC canine model for the study of human IBC and provide insight into this uncommon malignancy in dogs.

1. Introduction

Inflammatory mammary cancer (IMC) is a rare form of mammary cancer that affects dogs and women [in humans, IMC is known as inflammatory breast cancer (IBC)] and is characterized by a sudden onset and an aggressive clinical course (Lee and Tannenbaum, 1924; Susaneck et al., 1983;

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Pérez-Alenza et al., 2001). In the dog, the clinical appearance of IMC, which includes edema, hyperemia, firmness, skin ulceration and warmth, is similar to that described in women with IBC (Haagensen, 1956). Clinically observed inflammatory symptoms are a manifestation of dermal lymphatic involvement; tumor cell invasion in the dermal lymphatic channels is required for a pathological diagnosis (Susaneck et al., 1983; Tavassoli, 1999; Pérez-Alenza et al., 2001; Peña et al., 2003). Histologically, inflammatory infiltrate is not relevant in IMC cases (Peña et al., 2003). In the dog, the reported mean survival after diagnoses is less than 60 days when treated with either palliative therapy with broad-spectrum antibiotic and anti-inflammatory drugs (glucocorticoids or NSAIDs) (Pérez-Alenza et al., 2001; Clemente et al., 2009; Marconato et al., 2009) or chemotherapy (Clemente et al., 2009; de M. Souza et al., 2009). However, a better result has been achieved with an anti-cyclooxygenase 2 treatment; the mean survival for 7 piroxicam-treated dogs was 171 days (de M. Souza et al., 2009). In women, the recent median overall survival time is 4.2 years, which is low, despite new multimodality treatments (Gonzalez-Angulo et al., 2007).

Spontaneous canine IMC has been proposed as the best spontaneous animal model for studying IBC; moreover, IMC presents several advantages compared with IBC, such as higher prevalence, necropsy availability and larger samples, the majority of which have been acquired prior to chemotherapy (Peña et al., 2003). Still, little is known about the specific characteristics of canine IMC (Susaneck et al., 1983; Pérez-Alenza et al., 2001; Peña et al., 2003; Queiroga et al., 2005; Illera et al., 2006; Sánchez-Archidona et al., 2007; Clemente et al., 2009, 2010; de M. Souza et al., 2009; Marconato et al., 2009; Millanta et al., 2010).

Inflammatory breast cancer is a distinct form of locally advanced breast cancer, and the mechanisms responsible for the aggressive clinical evolution are incompletely understood; highly angiogenic, invasive and metastatic features are common to IBC and IMC (Jaiyesimi et al., 1992; Pérez-Alenza et al., 2001). In the dog, there is histopathological evidence that IMC tumor cells secrete lipids, which may be steroids (Peña et al., 2003). Moreover, the generation of microvascular channels by malignant tumor cells without endothelial cell participation (i.e., vasculogenic mimicry) has been reported in IBC (Shirakawa et al., 2001, 2002a,b,c, 2003; Kobayashi et al., 2002) and IMC (Clemente et al., 2010) and could be associated with high lymphangiogenic capacity and metastatic lymphangiotropism that are characteristic of this type of cancer (Clemente et al., 2010).

Cytokines are a large family of soluble, short-acting intercellular communication proteins that play important roles in inflammation, immunity and tissue homeostasis. Interleukins (ILs) are cytokines that mediate communication between leukocytes (Coico et al., 2003). A new hypothesis that is gaining acceptance suggests that some ILs could also play an important role in cancer as potential modulators of angiogenesis and leukocyte infiltration (Chavey et al., 2007). Inflammatory ILs, including IL-6 and IL-8, have been related to poor prognosis and can act as autocrine and paracrine growth factors in several types of human cancer (Angelo and Kurzrock, 2007). Still, little is known regarding the specific roles of the different ILs in cancer in general and in breast cancer in particular. Interleukin 1 (IL-1), which is a major pro-inflammatory cytokine, plays an important role in early tumor growth and metastasis by initiating the production of other mediators of invasiveness and angiogenesis (Li et al., 1995; Apte et al., 2006; Lewis et al., 2006). Interleukin-6 (IL-6) is another pro-inflammatory cytokine that promotes tumor growth by up-regulating antiapoptotic and angiogenic proteins in tumor cells, can stimulate the activity of enzymes that are involved in estrogen synthesis (Purohit et al., 2002) and it is also able to stimulate motility and decrease cell adhesion in breast cancer cell lines (Verhasselt et al., 1992; Asgeirsson et al., 1998; Arihiro et al., 2000). Interleukin-8, also termed CXCL8, could have a significant role in malignancy since it is involved in breast cancer invasion and angiogenesis (Lin et al., 2004). Interleukin-10, which is a down-regulator of immune responses (Coico et al., 2003), has been correlated with cancer associated-immunosuppression (Khong and Restifo, 2002).

The role of ILs in human breast cancer has been further investigated; ILs are known to have both tumor-promoting and inhibitory effects on breast cancer proliferation by binding specific receptors (Chavey et al., 2007). It is known that ILs can be produced either by inflammatory cells (tumour-associated macrophages and tumour-infiltrating lymphocytes) or by neoplastic mammary cells (Wilson and Balkwill, 2002; Freund et al., 2003). High IL-1, IL-6, IL-8 and IL-10 expression levels have been found in breast cancer patient sera and in breast tumor samples (Reed et al., 1992; Merendino et al., 1996; Kozlowski et al., 2003; Chavey et al., 2007; Knupfer and Preiss, 2007; Lyon et al., 2008), and elevated IL-6, IL-8 and IL-10 levels in breast cancer patient sera have also been associated with advanced clinical stage and poor prognosis (Merendino et al., 1996; Bachelot et al., 2003; Kozlowski et al., 2003; Benoy et al., 2004; Mettler et al., 2004; Derin et al., 2007).

In contrast, little is known about the role of these cytokines in human IBC, and a number of these results contradict one another. Interleukin-6 and IL-8 appear to be produced by IBC tumor cell lines (Van Golen et al., 2000). Others detected IL-1β and IL-8 in IBC-xenograft cells and in an IBC cell line; EIA-detected IL-8 levels were higher in IBC xenograft-derived murine sera and culture media compared with non-IBC xenograft-derived samples (Shirakawa et al., 2001). Bieche et al. (2004) found only higher IL-6 mRNA expression levels in IBC tumor samples compared with locally advanced breast cancer tumor samples; but no differences of the expression of IL-1α, IL-1β, IL-8, and IL-10 were found.

To date, only three studies have demonstrated the presence of several ILs in CMTs (Kim et al., 2010; Zuccari et al., 2011; Gelaleti et al., 2012). Using immunohistochemistry techniques, IL-1 and IL-6 immunoexpressions were higher in malignant and metastatic tumors compared with benign CMTs (Kim et al., 2010). Interleukin-8 has also been detected in CMTs; results are contradictory, though. It was initially suggested that IL-8 could play a protective role against canine mammary cancer (Zuccari et al., 2011), but later an association between high levels of serum IL-8 and a poor prognosis was reported by the same research