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NEOPLASTIC DISEASE

Immunohistochemical Expression of Cyclooxygenase-2 in Normal, Hyperplastic and Neoplastic Canine Lymphoid Tissues

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Summary

There is much interest in the potential use of selective inhibitors of cyclooxygenase (COX)-2 in combination with other cancer therapeutics. COX-2 is a key enzyme in prostaglandin synthesis and has been implicated in the pathogenesis of numerous canine and feline malignancies. There are few data on the potential role of COX-2 in the pathogenesis of canine lymphoma. The present study examined COX-2 expression in normal, hyperplastic and neoplastic canine lymphoid tissues. Immunohistochemical expression was evaluated in 12 samples of non-pathologically enlarged normal lymph nodes, 24 samples of hyperplastic lymph node and 44 samples of lymphoma (22 B-cell and 22 T-cell lymphomas). The labelling was scored semiquantitatively and a score of + 2 or + 3 was interpreted as overexpression. In hyperplastic lymph nodes only a few macrophages were COX-2-positive while six of the 44 lymphomas (13.6%; three B- and three T-cell lymphomas) overexpressed COX-2. These data provide a rationale for further investigation of COX-2 expression in canine lymphoma for prognostic, chemopreventive and chemotherapeutic purposes.

Keywords: COX-2; dog; lymphoid tissue; lymphoma

Introduction

Cyclooxygenases (COXs) are inflammatory regulators involved in the conversion of arachidonic acid to prostaglandins (PGs). The constitutively expressed isoform COX-1 contributes mainly to immediate PG generation and is responsible for homoeostasis, while the inducible COX-2 mediates delayed PG generation following various inflammatory stimuli or in the presence of neoplasia (Ghosh *et al.*, 2010). The COX metabolite PG H_2 (PGH_2) is then isomerized to PG E_2 (PGE_2) by terminal PGE_2 synthase enzymes. PGE_2 is produced by various tissues and has a broad range of biological roles including mediating pain, modulation of lymphocyte cytokine production and induction of interleukin (IL)-6 and haptoglobin, both of which are important regulators of angiogenesis (Fosslien, 2000). Furthermore, it has a key role in promoting tumourigenesis (Greenhough *et al.*, 2009).

Non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors have shown potential as immunotherapeutics for treatment of cancer. Several clinical studies have employed COX inhibitors in conjunction with chemotherapy or radiotherapy in order to eliminate or delay metastatic progression; such studies have also been performed in veterinary medicine (Spugnini *et al.*, 2005). COX-2 overexpression has been identified in canine and feline epithelial tumours (Tremblay *et al.*, 1999; Mohammed *et al.*, 1999, 2004; Pestilli de Almeida *et al.*, 2001; Khan *et al.*, 2001a, b; McEntee *et al.*, 2002; Dore *et al.*, 2003; Millanta *et al.*, 2006; Belshaw *et al.*, 2011) and in appendicular osteosarcoma (Mullins *et al.*, 2004; Millanta *et al.*, 2012), but until now the expression of COX-2 in canine lymphoma has not been definitively determined. Previous studies

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of canine lymphoid tissues revealed no COX-2 immunoreactivity in canine lymphoma or in normal lymph nodes (Mohammed *et al.*, 1999; Rodrigues *et al.*, 2011).

The primary aim of the present study was to evaluate further COX-2 expression in normal, hyperplastic and neoplastic canine lymphoid tissues. A secondary objective was to determine whether there is an association between COX-2 expression and the histopathological subtype of canine lymphoma.

Materials and Methods

Samples

Samples evaluated in this study included tissues from 44 cases of histologically confirmed canine lymphoma from the Tumour Registry of the Department of Veterinary Science, School of Veterinary Medicine, University of Pisa, Italy, and, as controls, 12 samples from non-pathologically enlarged normal canine lymph nodes collected during necropsy and 24 cases of canine lymph node reactive hyperplasia due to *Leishmania* spp. infection or chronic inflammatory reactions.

The tumour samples were collected between 2008 and 2010. Neoplastic tissues were collected at the time of diagnostic biopsy from 31 generalized B- or T-cell lymphomas and 13 cutaneous T-cell lymphomas. For all cases the following data were collected: age, breed, sex (without identifying neuter status) and tumour topography (Ponce *et al.*, 2010). Information regarding tumour staging was acquired from clinical notes. Each dog with lymphoma was staged according to the World Health Organization (WHO) clinical staging for lymphomas (Owen, 1980) on the basis of the results of physical examination and diagnostic workup, which included a complete blood cell count, serum biochemical analysis, thoracic radiography and abdominal ultrasound.

The median age of these dogs was 9.1 ± 2.3 years (range 3−15 years) and there were 16 females and 28 males. There were 29 purebred dogs (seven boxers, two dobermans, two German shepherd dogs, two shih tzus, two Labrador retrievers two schnauzers, one rottweiler, one Brie shepherd dog, one bulldog, one Great Dane, one cane corso, one golden retriever, one cocker spaniel, one shar pei, one Maremma sheepdog, one fox terrier, one Irish setter and one Weimaraner) and 15 mixed-breed dogs. All dogs presented with painless lymphadenopathy and had a widespread disease of stages III−IV.

The dogs with reactive hyperplasia of lymph nodes had a median age of 9.2 ± 2.9 years (range 1−13 years). There were 16 females and eight males. Eleven of the dogs were purebred (two German shepherd dogs, two Yorkshire terriers, two rottweilers, one Maremma sheepdog, one Belgian shepherd dog, one pointer, one Great Dane and one mastiff) and thirteen were mixed-breed dogs. The 12 dogs from which normal lymph node samples were collected were presented to the necropsy service of the Department of Veterinary Science, University of Pisa, Italy. These animals had no history of lymphoma or other chronic inflammatory diseases. There were eight male and four female dogs with a median age of 8.5 years (range 3−12 years). Five of these dogs were purebred (two English setters, one German shepherd dog, one Maremma sheepdog and one golden retriever) and seven were mixed-breed dogs.

Histological Evaluation

Biopsy specimens were fixed in 10% neutral buffered formalin at room temperature for 48 h, processed routinely and embedded in paraffin wax. Tissue samples from normal lymph nodes, collected during necropsy examination, were processed following the same procedures. Sections (4 μm) were stained with haematoxylin and eosin (HE) and examined by two pathologists (FM and AP) using a double-headed microscope to confirm the diagnosis, before immunophenotyping.

Lymphoma Immunophenotyping

Immunophenotyping was performed using antibodies directed against human antigens, but cross-reacting with the equivalent canine antigens. A polyclonal rabbit antibody against CD3 (Dako, Glostrup, Denmark; diluted 1 in 50) was used as a pan-T-cell marker (Ferrer *et al.*, 1992), a monoclonal antibody directed against CD79a (Santa Cruz Biotechnology, Santa Cruz, California, USA; diluted 1 in 50) and a rabbit polyclonal antibody against CD20 (Thermo Scientific, Freemont, California, USA; diluted 1 in 400) were used as pan-B-cell markers (Jones *et al.*, 1993). A standard avidin−biotin−immuno-peroxidase procedure was used (Fournel-Fleury *et al.*, 1995). Immunophenotyping results were evaluated by the same pathologists (FM, AP) independent of the morphological study.

Classification

Classification of the 44 cases was initially based on cellular morphology and immunophenotype according to the updated Kiel classification adapted to account for specific canine subtypes (Fournel-Fleury *et al.*, 2002; Ponce *et al.*, 2010). B-cell morphologies that did not fit the Kiel classification system, particularly marginal zone lymphomas, were described according to the

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