J. Comp. Path. 2016, Vol. ■, 1–7

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Immune Cell Infiltration in Feline Meningioma

R. McBride*, E. A. Sloma*, H. N. Erb† and A. D. Miller*

** Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY and † Department of Population Medicine and Diagnostic Sciences (HNE), Cornell University College of Veterinary Medicine, Ithaca, NY, USA*

Summary

Meningioma is the most common primary brain tumour in the cat. Although most of these tumours are considered to be benign based on histological characteristics, little is known regarding microenvironmental changes associated with the tumour. The characterization of the immune-cell infiltrate in human and canine meningiomas has been described; however, there are no data regarding the cat equivalent. Seventeen formalin-fixed, paraffin wax-embedded samples of feline meningioma were evaluated by light microscopy and immunohistochemistry (IHC) for expression of CD3, Pax5, Iba-1, HLA-DR, MAC387, CD163 and Ki67. Variable immune cell infiltrates were seen in every case. All tumours had moderate numbers of infiltrating $CD3^+$ T lymphocytes. Seven of 17 tumours (41%) had infiltrating $PAX5^+$ B lymphocytes that were often dispersed randomly throughout the neoplasm. Macrophage infiltration was abundant in all tumours with widespread immunoreactivity for Iba-1 and HLA-DR. Most tumours (15/17; 88%) had infiltration by $MAC387^+$ macrophages; however, the number of infiltrating cells per ×400 field varied widely (from 0 to 57). Thirteen of 17 tumours (76%) had infiltration by $CD163^+$ macrophages; however (similar to the MAC387 IHC), several tumours had numerous infiltrating cells. There was a potential weak negative rank correlation between the counts of CD3 and $Ki67^+$ cells ($r_{Sp} = -0.57$; $P = 0.018$); however, no other rank correlations could be established between types of infiltrating immune cells (all other $P \geq 0.10$). This study establishes evidence for a robust population of immune cells in feline meningioma and indicates that further study is needed to better understand the role of these cells with respect to tumour progression and post-surgical outcome.

Keywords: cat; immune response; immunohistochemistry; meningioma

Introduction

In cats, meningiomas are the most common primary brain tumour. They arise from the arachnoid layer of the meninges and comprise up to 59% of intracranial neoplasia in the cat (Zaki and Hurvitz, 1976; Troxel *et al.*, 2003). Meningiomas are most prevalent in older cats, with a median age of 12 years; however, there is no known sex predilection (Troxel *et al.*, 2003; Cameron *et al.*, 2015). Although domestic short-haired cats are overrepresented, this may be due to the preponderance of this breed in the general cat population and therefore not reflective of true increased incidence (Troxel *et al.*, 2003; Motta *et al.*, 2012). Compared with meningioma development in other species, cats are at increased risk of developing multiple concurrent tumours, including up to 19% of cats in one report (Zaki and Hurvitz, 1976; Forterre *et al.*, 2007).

Histologically, feline meningiomas are composed typically of spindle-shaped, elongate cells often forming whorls that are embedded in a collagen-rich matrix with variable linear to focal mineralization (psammoma bodies), necrosis and cholesterol clefts (Vandevelde *et al.*, 2012). The most common histological subtypes of feline meningiomas are psammomatous, transitional and meningothelial (Forterre *et al.*, 2007; Cameron *et al.*, 2015). Factors that control

Correspondence to: A. D. Miller (e-mail: adm10@cornell.edu).

0021-9975/$ - see front matter

http://dx.doi.org/10.1016/j.jcpa.2016.10.011

Please cite this article in press as: McBride R, et al., Immune Cell Infiltration in Feline Meningioma, Journal of Comparative Pathology (2016), http://dx.doi.org/10.1016/j.jcpa.2016.10.011

malignancy in feline meningiomas are not clear. Anatomical sites of increased frequency include the meninges of either the falx cerebri or tentorium cerebelli and the tela choroidea of the third ventricle (Troxel *et al.*, 2003; Motta *et al.*, 2012). Currently, no accepted grading system exists for feline meningioma; however, based on the histological and clinical features of these tumours, most are assumed to be low grade.

In man, brain tumour development, maintenance and progression can be linked to alterations in the immune system. A robust infiltrate of lymphocytes and macrophages is associated with human meningiomas and a single study in dogs revealed similar findings (Louis *et al.*, 2007; Boozer *et al.*, 2012). Higher-grade tumours tend to have increased numbers of infiltrating macrophages; however, the cause and effect of this increase is not known (Boozer *et al.*, 2012). Disease progression may be mediated, in part, by the anti-tumour and pro-tumour effects of the infiltrating macrophages; the macrophages can be stratified into different subtypes based on which molecules induce them and which proteins they express. M1 macrophages are induced by a variety of cytokines including interferon (IFN)-γ and granulocyte—monocyte colony stimulating factor (GM-CSF) (Domingues *et al.*, 2016). Expression of MAC387 (calprotectin) is an indication of an M1 (pro-inflammatory) macrophage subtype (Soulas *et al.*, 2011). This macrophage subtype secretes a variety of pro-inflammatory cytokines, produces a variety of reactive oxygen species and is generally viewed as an anti-tumourigenic macrophage (Soulas *et al.*, 2011). Expression of CD163 and other surface receptors is associated with M2 macrophages that are involved in an anti-inflammatory response which, in turn, confers a pro-tumourigenic effect during tumourigenesis and progression (Prosniak *et al.*, 2013). Differences between M1 and M2 macrophage infiltration in feline meningioma are not known. Similarly, the subpopulation of lymphocytes that infiltrate a neoplasm play a role in tumour progression in man; however, the field of immunopathology has only recently been explored in veterinary cancers (e.g. marked infiltration of cytotoxic T cells is associated with anti-tumour effects) (Fang *et al.*, 2013; Sloma *et al.*, 2015).

Due to the differences that macrophages and lymphocytes have in generating the immune response, we sought to define the immune cell infiltrates associated with feline meningioma. We performed a retrospective study of 17 cases of feline meningioma to determine the presence, distribution and correlation in counts of B and T lymphocytes and macrophage subtypes.

Materials and Methods

The pathology database at Cornell University, College of Veterinary Medicine, was searched for cases of meningioma in the cat between 2007 and 2015. All cases except one were surgical biopsy specimens and were all analyzed by one of the authors (ADM). Tissues were collected, fixed in 10% neutral-buffered formalin, processed routinely and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE).

To characterize the immune cell infiltrate, the following primary antibodies were used: anti-CD3 (T lymphocytes), anti-Pax5 (directed at a transcription factor for B-lymphocyte development), anti-ionized calcium-binding adapter protein-1 (Iba-1) (directed at a calcium-binding protein expressed in macrophages), anti-HLA-DR (directed at class II molecules of the major histocompatibility complex expressed on tissue macrophages and microglia), MAC387 (directed at the cytoplasmic antigen calprotectin found in circulating monocytes and macrophages matured from blood monocytes), CD163 (directed at a scavenger receptor on subsets of macrophages) and Ki67 (a marker of cell proliferation). Sections were dewaxed in xylene, hydrated in graded ethanols and subsequently blocked with 3% H_2O_2. Pretreatment for Iba-1, HLA-DR, MAC387 and Ki67 labelling was microwaving for 20 min in 0.1 M citrate buffer followed by 20 min of cooling. Pretreatment for CD163 labelling was 10 min of proteinase K digestion. All steps were followed by a Tris-buffered saline wash. Prior to the application of the primary antibodies, all slides were treated with an avidin—biotin protein block (Invitrogen, Carlsbad, California, USA) and serum-free protein block (Dako, Carpinteria, California, USA) for 10 min at room temperature. The sections were incubated with primary antibody. The incubation times, dilution, clone and manufacturer for each antibody are listed in Supplementary Table 1. Secondary antibodies were biotinylated horse anti-mouse (Vector Laboratories, Burlingame, California, USA) except for Iba-1 (biotinylated goat anti-rabbit, Vector Laboratories), diluted at 1 in 200 and applied for 30 min at room temperature. Tertiary reagent was Vectastain ABC Elite reagent (Vector Laboratories) incubated for 30 min at room temperature. CD3 and Pax5 labelling were performed using the Leica BOND MAX immunohistochemistry (IHC) system using standardized protocols. For these two antibodies, antigen retrieval was performed with Novocastra Bond Epitope Retrieval Solution 2. CD3 labelling was 'visualized' using 3,3′-diaminobenzidine (DAB)

Please cite this article in press as: McBride R, et al., Immune Cell Infiltration in Feline Meningioma, Journal of Comparative Pathology (2016), http://dx.doi.org/10.1016/j.jcpa.2016.10.011