J. Comp. Path. 2018, Vol. 160, 15–22

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Immunohistochemical Characterization of Immune Cell Infiltration in Feline Glioma

D. R. Rissi*, B. F. Porter†, C. E. Boudreau‡, P. M. Krimer* and A. D. Miller§

** Department of Pathology and Athens Veterinary Diagnostic Laboratory, University of Georgia College of Veterinary Medicine, Athens, Georgia, † Department of Veterinary Pathobiology, ‡ Small Animal Clinical Sciences, Texas A&M University College of Veterinary Medicine and Biomedical Sciences, College Station, Texas and § Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, New York, USA*

Summary

The relationship between inflammatory cells and tumour biology has been defined in many human intracranial neoplasms, but it is relatively poorly characterized in veterinary medicine. The aim of this study was to define the immune cell infiltration in cases of feline glioma and its possible association with tumour morphology and type. A retrospective search identified 18 gliomas that met inclusion criteria. Tumours were subjected to immunohistochemistry (IHC) for CD3, CD20, Iba1, MAC387 and factor VIII-related antigen. For each antibody, the number of labelled cells was counted in 10 high-power (×400) fields and a cumulative score for each antibody was generated. Intratumoural and peritumoural $CD3^+$ T lymphocytes were observed in all cases and occurred primarily within perivascular spaces and rarely around areas of necrosis or leptomeningeal spread. Perivascular $CD20^+$ B lymphocytes were detected in 12/18 (67%) cases and occurred within and around tumours and near areas of leptomeningeal spread. MAC387 immunoreactivity highlighted intravascular monocytes in 9/18 (50%) cases, but failed to highlight tumour-associated macrophages. Intratumoural and peritumoural Iba1 immunoreactivity was observed in all cases, with increased overall intensity around areas of necrosis and leptomeningeal spread. Intratumoural and peritumoural factor VIII-related antigen immunoreactivity was also detected in all cases and was concentrated in areas of microvascular proliferation and necrosis. No significant associations were found between IHC scores for immune cells (i.e. lymphocytes and macrophages) and tumour morphology and type. Average factor VIII reactivity was higher in astrocytomas than oligodendrogliomas ($P = 0.003$).

Keywords: brain; cat; glioma; tumour microenvironment

Introduction

Glioma constitutes a group of neoplasms (i.e. oligodendroglioma, astrocytoma and ependymoma) that arise from glial cells of the central nervous system (CNS). In cats, gliomas are second only to meningioma as the most common primary CNS tumours (Troxel *et al.*, 2003, 2004; Rissi and Miller, 2017). Gliomas typically affect adult cats, with no sex or breed predisposition and occur primarily in the brain with less frequent involvement of the spinal cord (Troxel *et al.*, 2003; Hammond *et al.*, 2014; Rissi and Miller, 2017). Astrocytoma appears to be the most common glioma in cats, followed by ependymoma, oligodendroglioma and other less common subtypes such as gliomatosis cerebri, but proportions vary in different studies (Hammond *et al.*, 2014; Rissi and Miller, 2017). Tumour grading is based on the World Health Organization grading system for human gliomas and relies on

Correspondence to: D.R. Rissi (e-mail: rissi@uga.edu).

0021-9975/$ - see front matter
https://doi.org/10.1016/j.jcpa.2018.02.003

specific morphological features, such as cell density and pleomorphism, mitotic activity and the presence of necrosis and microvascular proliferation (Hammond *et al.*, 2014; Louis *et al.*, 2016; Rissi and Miller, 2017).

The glial tumour microenvironment can be influenced by many cell types, including glial cells, neural cells, stem cells and inflammatory cells (Sowers *et al.*, 2014). The relationship between the immune cell population and tumour type, behaviour and prognosis has been well characterized in human gliomas (Charles *et al.*, 2011; Lorger, 2012; Sowers *et al.*, 2014; Domingues *et al.*, 2016). Glioblastoma, the most common and malignant glioma in humans, has a median survival time of 15 months after diagnosis, even after multimodal treatment including surgical resection, radiation therapy and chemotherapy (Thomas *et al.*, 2012). A more recent treatment modality, immunotherapy, requires a detailed knowledge of the intratumoural and peritumoural immune cell populations within the tumour microenvironment (Thomas *et al.*, 2012). Immunotherapy seeks to regulate the host immune response for more effective tumour regression. Immunotherapy methods include indirect stimulation of the immune system by the administration of specific antibodies, cytokines or immune effector cells (i.e. passive immunotherapy) or direct stimulation of the immune system by the administration of specific tumour antigens (i.e. active immunotherapy) (Thomas *et al.*, 2012). Immunotherapy has been used in a wide variety of human cancers, including those of the breast, prostate, bladder, lung, colorectum and lymphoid system (Dillman, 2011). However, the relative immune privilege status of the CNS and the immunosuppression caused by high-grade gliomas makes immunotherapy usage challenging in these patients (Thomas *et al.*, 2012). Nevertheless, clinical trials are currently underway and encouraging results have been observed (Dillman, 2011; Thomas *et al.*, 2012).

Advanced and often less invasive surgical procedures have been developed for intracranial tumour resection in companion animals (Troxel *et al.*, 2003; Klopp and Rao, 2009). However, more infiltrative tumours still require traditional surgery and adjunctive therapy such as radiation and chemotherapy to improve patient wellbeing and increase overall post-surgical survival (Troxel *et al.*, 2003; Klopp and Rao, 2009). Studies using immunotherapy in canine brain tumours have shown tumour reduction and clinical improvement. However, the low number of patients used in these protocols precludes knowing if these findings are applicable to glioma patients as a whole (Ingram *et al.*, 1990; Pluhar *et al.*, 2010; Andersen *et al.*, 2013). Further development of CNS tumour immunotherapy in companion animals will require better understanding of the role of the immune system in tumour biology. Only a few veterinary studies have characterized the immune cell population in primary CNS tumours and none of these have focused on feline glioma (Boozer *et al.*, 2012; Sloma *et al.*, 2015; McBride *et al.*, 2016). The current study characterizes the immune cell population in cases of feline glioma and examines possible correlations between type and degree of inflammation and tumour morphology and type.

Materials and Methods

Cases of feline glioma were identified retrospectively from the archives of the University of Georgia Athens Veterinary Diagnostic Laboratory (Athens, Georgia), the New York State Animal Health Diagnostic Center, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine (Ithaca, New York) and the Department of Veterinary Pathobiology, Texas A&M University College of Veterinary Medicine (College Station, Texas) between January 2000 and December 2016. Eighteen cases were identified. Cases 1–5, 7–10 and 18 had been diagnosed previously based on histopathology and immunohistochemistry (IHC) for glial fibrillary acidic protein (GFAP) and oligodendrocyte lineage transcription factor 2 (Olig2) (Rissi and Miller, 2017). Similarly, histopathology and IHC for GFAP and Olig2 was conducted for diagnostic confirmation on cases 6 and 11–17. All cases were reviewed by the first author and after diagnostic confirmation and grading according to the 2016 World Health Organization Classification of Tumours of the Central Nervous System (Louis *et al.*, 2016), cases 1–18 were immunolabelled for CD3 (directed against T lymphocytes; rabbit polyclonal antiserum, 1 in 1,000 dilution for 60 min; Dako, Carpinteria, California, USA; catalogue number A05452), CD20 (directed against B lymphocytes; rabbit polyclonal antiserum, 1 in 2, 000 dilution for 90 min; Biocare, Pacheco, California, USA; catalogue number 121R-18), Iba1 (directed against macrophages and microglia; rabbit polyclonal antiserum, 1 in 8,000 dilution for 60 min, Wako, Richmond, Virginia, USA; catalogue number 019-19741), MAC387 (directed against monocytes and macrophages originating from blood monocytes; mouse monoclonal antibody, 1 in 500 dilution for 60 min, Dako, catalogue number M0747) and factor VIII-related antigen (FVIIIRA, directed against endothelium; rabbit polyclonal antiserum, ready to use for 60 min, Cell Marque, Rocklin, California, USA;