J. Comp. Path. 2017, Vol. 156, 147–151

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

INFECTIOUS DISEASE

Glial Changes and Evidence for Apoptosis in the Brain of Cats Infected by *Cytauxzoon felis*

L. L. Clarke*, P. M. Krimer† and D. R. Rissi†

** Department of Pathology, College of Veterinary Medicine, The University of Georgia, Athens, GA and † Athens Veterinary Diagnostic Laboratory, College of Veterinary Medicine, The University of Georgia, Athens, GA, USA*

Summary

Ischaemic neuropathological changes associated with *Cytauxzoon felis* infection in cats have been reported recently. This paper describes the associated glial changes and the evidence for apoptosis in the brain of cats infected naturally by *C. felis*. Sections of brain from eight affected cats and eight age- and sex-matched control cats were evaluated by immunohistochemistry for expression of glial fibrillary acidic protein, CD18 and cleaved caspase-3. Vascular changes in the leptomeninges and parenchyma, the number of positive astrocytes and phagocytic cells (microglia or macrophages) and the average astrocytic cytoplasmic area and number and length of astrocytic processes were quantified, and a mean value for the grey and white matter in both groups was generated. Astrocytic hyperplasia (astrogliosis) and phagocytic cell hyperplasia were detected in all affected cats. Immunoexpression of cleaved caspase-3 was detected in intravascular and perivascular macrophages in the leptomeninges and, less often, in the grey and white matter in all affected cats. Four cats with encephalomalacia had additional cytoplasmic immunolabelling of phagocytic cells around the necrotic foci and macrophages and cell debris within the areas of necrosis. These results support the role of an extensive reaction of the brain tissue to hypoxia–ischaemia and a potential role of apoptosis in the neuropathogenesis of *C. felis* infection in cats.

Keywords: cat; *Cytauxzoon felis*; hypoxia–ischaemia; neuropathology

Introduction

Due to its high metabolic demand for energy, the brain is susceptible to subtle changes in blood and oxygen supply (Busl and Greer, 2010). Following a hypoxic–ischaemic insult, disruptions in the mitochondrial oxidative phosphorylation system may occur before morphological changes are detected and these early, subtle changes should be taken into consideration when evaluating the clinical signs of a patient with neurological disease (Suchadolskiene *et al.*, 2014). The neuropathological changes in cats infected naturally by *Cytauxzoon felis* have been described and are consistent with a hypoxic–ischaemic insult secondary to vascular occlusion by schizont-laden macrophages (Clarke and Rissi, 2015). Parenchymal changes consist of vacuolation of the grey and white matter, with microhaemorrhages and random areas of necrosis (Clarke and Rissi, 2015). Although distinct areas of gliosis are present throughout the grey and white matter, a more widespread hypercellularity, which is presumably attributed to a diffuse glial response, is also observed.

We hypothesized that diffuse gliosis could represent an early reaction to hypoxia–ischaemia and contribute to the neuropathogenesis of *C. felis* infection. In addition, reactive astrocytes may respond to hypoxic–ischaemic insult by releasing pro-apoptotic signals that can contribute to further brain damage via apoptosis (Sidoryk-Wegrzynowicz *et al.*, 2011); thus we also theorized that apoptosis could contribute to the development of the degenerative changes in the neuroparenchyma of affected cats.

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

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2016.11.268

Materials and Methods

Sections of brain from eight cats infected naturally by *C. felis* and eight age- and sex-matched control cats that died of causes not related to central nervous system disease were evaluated. All sections consisted of mid-telencephalon at the level of the thalamus and were scanned at low power (×200) to define areas of grey and white matter before detailed histological and immunohistochemical evaluation was performed. Vascular changes were evaluated in five randomly selected high-power fields (×400) of the grey matter, white matter and leptomeninges. The degree of vascular occlusion by schizont-laden macrophages was assessed on haematoxylin and eosin (HE)-stained sections (Snider *et al.*, 2010). Vascular occlusion was scored from 0 to 3, where 0, no occlusion; 1, 1–39% of vessels occluded; 2, 40–69% of vessels occluded; and 3, 70–100% of vessels occluded. The degree of perivascular inflammation was scored from 0 to 3, where 0, no inflammation; 1, 1–2 layers of inflammatory cells; 2, 3–4 layers of inflammatory cells; and 3, >5 layers of inflammatory cells. Tissue sections were evaluated by immunohistochemistry (IHC) for expression of glial fibrillary acidic protein (GFAP) using a mouse monoclonal antibody (Biogenex, Fremont, California, USA) at a dilution of 1 in 4,000 and applied for 60 min; CD18 using a mouse monoclonal antibody (P. F. Moore, University of California, Davis, California, USA) at a dilution of 1 in 10 and applied for 60 min; and cleaved caspase-3 using a rabbit polyclonal antibody (Biocare, Concord, California, USA) at a dilution of 1 in 100 and applied for 90 min. Negative controls consisted of normal brain tissue and positive controls consisted of normal brain tissue (GFAP) and tonsil (CD18 and cleaved caspase-3). The number of GFAP-immunopositive astrocytes and CD18-immunopositive phagocytic cells (microglia and macrophages) was determined for both groups using Adobe Photoshop CS6 Extended™. For that purpose, 10 microphotographs from randomly selected high-power fields (×400) of grey matter (five images) and white matter (five images) in each case were evaluated. Endothelial cells were not considered when evaluating CD18 IHC.

The average astrocytic cytoplasmic area and the number and length of astrocytic cytoplasmic processes were quantified automatically using Image-Pro Plus™ (Media Cybernetics, Rockville, Maryland, USA). Mean values for each of these parameters was generated by the software after evaluation of 10 microphotographs from randomly selected high-power fields (×400) of grey matter (five images) and white matter (five images) in each case. All data sets were subjected to a two-sample *t*-test to compare changes in the grey and white mater between affected cats and control cats. $P < 0.05$ was considered significant.

Univariate and multivariate regression analysis was performed using total summed values to examine potential relationships between parasitic load, perivascular inflammation and number of astrocytes and phagocytic cells. All analyses were performed using Minitab® 15 (Minitab Inc., State College, Pennsylvania, USA). Cleaved caspase-3 IHC was examined by evaluation of the entire section and was conducted by assessing the vascular component (including leptomeningeal, parenchymal and choroid plexus) and the grey and white matter. The presence or absence of cleaved caspase-3 immunolabelling, as well as the location of the labelling and the involved cell type, were determined.

Results

All cats exhibited some degree of vascular occlusion and perivascular inflammation (Fig. 1). Morphometric results (Supplementary Figs. 1 and 2) revealed astrogliosis and an increased number of phagocytic cells throughout the examined areas of grey and white matter in all affected cats (Fig. 2). The astrocytic cytoplasmic area and number and length of astrocytic cytoplasmic processes were not significantly different to those parameters in the control group. Regression analysis did not find any significant relationship between parasitic load, perivascular inflammation and the number of astrocytes or phagocytic cells. Areas of positive immunolabelling for cleaved caspase-3 (Fig. 3) were present in all affected cats and absent

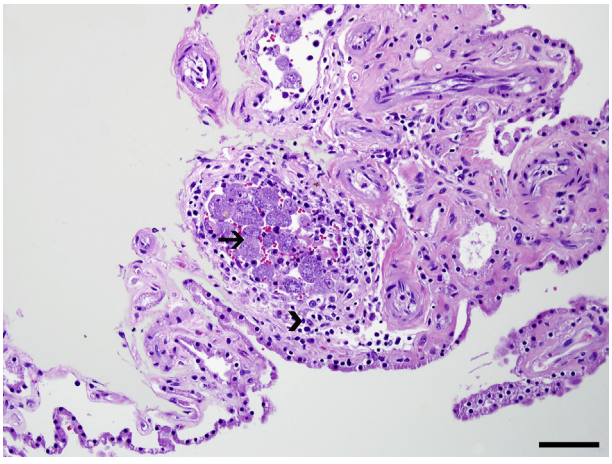

Fig. 1. Leptomeningeal vessel (case 4) almost completely occluded (occlusion score 3) by schizont-laden macrophages (arrow) and surrounded by a few layers of lymphocytes and plasma cells (arrowhead, perivascular inflammation score 2). HE. Bar, 100 μm.