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Basic Science

Dominance of chemokine ligand 2 and matrix metalloproteinase-2 and -9 and suppression of pro-inflammatory cytokines in the epidural compartment after intervertebral disc extrusion in a canine model

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Abstract

BACKGROUND CONTEXT: In canine intervertebral disc (IVD) disease, a useful animal model, only little is known about the inflammatory response in the epidural space.

PURPOSE: To determine messenger RNA (mRNA) expressions of selected cytokines, chemokines, and matrix metalloproteinases (MMPs) qualitatively and semiquantitatively over the course of the disease and to correlate results to neurologic status and outcome.

STUDY DESIGN/SETTING: Prospective study using extruded IVD material of dogs with thoracolumbar IVD extrusion.

PATIENT SAMPLE: Seventy affected and 13 control (24 samples) dogs.

OUTCOME MEASURES: Duration of neurologic signs, pretreatment, neurologic grade, severity of pain, and outcome were recorded. After diagnostic imaging, decompressive surgery was performed.

METHODS: Messenger RNA expressions of interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor (TNF), interferon (IFN)γ, MMP-2, MMP-9, chemokine ligand (CCL)2, CCL3, and three housekeeping genes was determined in the collected epidural material by Panomics 2.0 QuantiGene Plex technology. Relative mRNA expression and fold changes were calculated. Relative mRNA expression was correlated statistically to clinical parameters.

RESULTS: Fold changes of TNF, IL-1β, IL-2, IL-4, IL-6, IL-10, IFNγ, and CCL3 were clearly downregulated in all stages of the disease. MMP-9 was downregulated in the acute stage and upregulated in the subacute and chronic phase. Interleukin-8 was upregulated in acute cases. MMP-2 showed mild and CCL2 strong upregulation over the whole course of the disease. In dogs

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with severe pain, CCL3 and IFNγ were significantly higher compared with dogs without pain (p=.017/.020). Dogs pretreated with nonsteroidal anti-inflammatory drugs revealed significantly lower mRNA expression of IL-8 (p=.017).

CONCLUSIONS: The high CCL2 levels and upregulated MMPs combined with downregulated T-cell cytokines and suppressed pro-inflammatory genes in extruded canine disc material indicate that the epidural reaction is dominated by infiltrating monocytes differentiating into macrophages with tissue remodeling functions. These results will help to understand the pathogenic processes representing the basis for novel therapeutic approaches. The canine IVD disease model will be rewarding in this process.

Keywords: Epidural space; Microarray; IL-8; MCP-1; CCL2; MMP

Introduction

Animal models of spontaneous intervertebral disc (IVD) degeneration have been reported in sand rats [1], pintail mice [2], baboons [3], and dogs [4,5]. Among these models, spontaneous IVD degeneration with subsequent herniation occurs commonly in dogs only, where it resembles the pathologic features of the human disease. Therefore, canine intervertebral disc disease (IVDD) is considered to be a useful animal model [6].

Intervertebral disc extrusion in dogs has an overall prevalence of 2% occurring commonly in chondrodystrophic but also in other breeds [7–10]. Relatively limited work has been performed on the pathophysiology of secondary reactions within the epidural space after IVD extrusion in dogs [11–13] and in humans [14–21]. Although it can be assumed that the inflammatory response in the epidural space plays an important role in the course and outcome of IVD extrusions [12–21], an acute inflammatory reaction could perhaps aggravate the clinical signs because of swelling of the inflamed extradural tissue causing further cord compression or that in case of dural injury inflammatory mediators may directly affect nervous tissues [22]. In reverse, phagocytosis and other inflammatory mechanisms that attenuate the space-occupying effect of extruded disc material within the epidural space may have a beneficial effect on the course of the disease [23,24].

The nature of this inflammatory process in the epidural compartment after IVD extrusion has been controversially discussed in humans and in dogs. Some reports indicate an adaptive immune response with infiltration of mainly T- and B-cells [12,25], whereas others demonstrated predominantly monocytes/macrophages [13,26–28] and/or multinucleated giant cells indicative of a foreign body reaction [12]. Several studies have been done on the expression of inflammatory mediators in epidural material after IVD herniation in humans [16–18,29,30] but none in dogs. Our own recent study in canine IVDD suggested an innate immune response in the epidural space with neutrophils and macrophages and extremely little evidence of an adaptive immune response [13]. To further understand and characterize the epidural inflammatory process in canine IVDD in the present study, we determined the expressions of selected canine cytokines, chemokines, and matrix metalloproteinases (MMPs) at the messenger RNA (mRNA) level in the epidural space after naturally occurring IVD extrusion. Because of the predominance of an innate immune response with neutrophils and macrophages as main contributors, we focused on classical pro-inflammatory cytokines like interferon (IFN)γ, tumor necrosis factor (TNF), interleukin (IL)-1b, IL-2, IL-6, and anti-inflammatory cytokines like IL-4 and IL-10. Owing to the monocytic infiltration, monocyte/macrophage-derived chemokines like chemokine ligand (CCL)2 and CCL3 were determined. Matrix metalloproteinase-2 and MMP-9 were selected because they have been commonly described in association with IVDD and are responsible for matrix degradation. Second, these two MMPs are well known in their function and were readily available in the canine microarray. Because of the lack of an adapted immune response with only a few T- and B-lymphocytes on histologic examination of extruded disc material, we did not determine further lymphocyte-specific cytokines or chemokines. Additionally, in an attempt to define potentially useful molecular markers for clinical use, the findings were correlated to age, severity of neurologic deficits, pain, medical pretreatment, and outcome.

Materials and methods

In this prospective study, extruded disc material from dogs with surgically confirmed thoracolumbar IVD extrusion presented from May 2011 to June 2013 at the Veterinary Teaching Hospitals (VTHs) of the Universities of Bern and Ghent were used.

Inclusion criteria were as follows: well-documented records of onset of clinical signs, neurologic findings, pretreatment, and diagnosis and course of the disease; a neurologic examination was done within 12 hours before spinal surgery; and extruded disc material with a minimal sample weight of 10 mg was stored according to a standardized protocol [14]. Additionally, IVD samples were collected from dogs that died or were euthanized without signs of a central nervous system disease and had not received any anti-inflammatory treatment in the last 4 weeks before death or euthanasia.

Clinical data

Breed, age, gender, duration of neurologic signs, medical pretreatment (steroids, nonsteroidal anti-inflammatory