Cerebrospinal Fluid Inflammatory Cytokines and Chemokines in Naturally Occurring Canine Spinal Cord Injury

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Abstract

Canine intervertebral disk herniation (IVDH) is a common, naturally occurring form of spinal cord injury (SCI) that is increasingly being used in pre-clinical evaluation of therapies. Although IVDH bears critical similarities to human SCI with respect to lesion morphology, imaging features, and post-SCI treatment, limited data are available concerning secondary injury mechanisms. Here, we characterized cerebrospinal fluid (CSF) cytokines, and chemokines in dogs with acute, surgically treated, thoracolumbar IVDH ($n=39$) and healthy control dogs ($n=21$) to investigate early inflammatory events after SCI. A bioplex system was used to measure interleukin (IL)-2, -6, -7, -8, -10, -15, and -18, granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN- γ), keratinocyte chemoattractant (KC)-like protein, IFN- γ -inducible protein-10, monocyte chemotactic protein 1 (MCP-1), and tumor necrosis factor alpha. Cytokine and chemokine concentrations in the CSF of healthy and SCI dogs were compared and, in SCI dogs, were correlated to the duration of SCI, behavioral measures of injury severity at the time of sampling, and neurological outcome 42 days post-SCI as determined by a validated ordinal score. IL-8 concentration was significantly higher in SCI cases than healthy controls ($p=0.0013$) and was negatively correlated with the duration of SCI ($p=0.042$). CSF MCP-1 and KC-like protein were positively correlated with CSF microprotein concentration in dogs with SCI ($p<0.0001$ and $p=0.004$). CSF MCP-1 concentration was negatively associated with 42-day postinjury outcome ($p<0.0001$). Taken together, these data indicate that cytokines and chemokines present after SCI in humans and rodent models are associated with SCI pathogenesis in canine IVDH.

Key words: inflammation; models of injury; neural injury

Introduction

THE MEDICAL COMMUNITY has been unable to develop an intervention that improves clinical outcome in spinal cord injury (SCI) despite over 60 phase II and III human clinical trials.¹ Possible reasons for failed therapeutic trials may include limited regenerative capacity of the central nervous system, comorbidities of affected patients, lack of blinding/randomization in pre-clinical trials, and limitations of animal model systems.^{1–4} In particular, rodent contusion models have been criticized because the spinal cord of injured animals is small, strains are highly inbred, injuries are stereotypical with respect to severity and timing, and the models do not mimic the rehabilitative treatments used in humans after SCI.^{1,3,5}

Canine intervertebral disk herniation (IVDH) is a common, naturally occurring form of contusive/compressive SCI observed in pet dogs.^{6,7} Canine IVDH has critical similarities to human SCI, including lesion morphology, imaging features, and surgical/rehabilitative treatment modalities.⁷ A small group of studies has

demonstrated that inflammatory mechanisms may play a critical role in canine IVDH-associated SCI.^{8–11} In particular, in acute canine SCI, resident microglial activation is prominent as is up-regulation of matrix metalloproteinase 9, release of myelin basic protein, and overproduction of interleukin (IL)-6 and IL-8 messenger RNA (mRNA).^{9–12} In order to further develop this model system, a broader understanding of inflammatory mechanisms after SCI is needed, particularly if pathways are to be targeted in pre-clinical trials.

Cerebrospinal fluid (CSF) is an avenue to evaluate cytokine and chemokine expression after SCI in living patients. The critical advantages of this approach, in contrast to traditional histological studies, include the ability to correlate cytokine and chemokine concentration with outcome variables and, in the setting of clinical trials, to utilize cytokines and chemokines as a means to stratify populations and/or monitor response to therapy.¹³ Recent studies have evaluated a broad range of inflammatory cytokines and chemokines in the CSF of humans with SCI and have shown increases in IL-6, IL-8, and monocyte chemotactic protein 1 (MCP-1)

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concentrations, compared to healthy individuals.^{13–16} The aim of the study reported on here was to evaluate a relatively large group of cytokines and chemokines that reflect both innate and adaptive immune responses in the CSF of dogs with acute IVDH. We hypothesized that 1) CSF cytokines predominantly reflecting early inflammatory events (especially IL-6 and IL-8) would be increased in dogs with IVDH, relative to healthy control dogs, and that 2) CSF cytokine and chemokine expression would correlate to the duration of injury, functional status at the time of sampling, and 42-day post-SCI motor outcome.

Methods

Sample-size considerations

Sample size was estimated before the initiation of this study using a formula available in a commercially available software package (S-PLUS statistical software Version 8.2; TIBCO Software, Inc., Seattle, WA). For all estimates of sample size, we assumed statistical power of 80%, a significance level of 5%, and normal distribution of data and residual errors. The study was powered to address our central hypothesis: that inflammatory cytokines would be elevated after canine SCI. Expected IL-6 and IL-8 concentrations were used because these cytokines have been previously shown to be robustly increased after SCI in humans.^{13,16} Based on published distributions of CSF cytokine concentrations in humans with SCI,¹³ we estimated that 5 control dogs and 10 SCI dogs were needed to detect the assumed magnitude of difference between groups in IL-6 concentration. Additionally, 14 control dogs and 28 SCI dogs were needed to detect assumed differences between groups in IL-8 concentration. We elected to use all canine CSF samples that met inclusion and exclusion criteria listed below (39 SCI and 21 control dogs) to improve precision of estimates, because CSF IL-6 and IL-8 have not been assessed in dogs previously and because our assay for determining these cytokines differed from that used in humans with SCI.

Spinal cord injured and healthy control dogs

All animal procedures were approved by the Texas A&M University (College Station, TX) Institutional Animal Care and Use Committee (AUP 2007-115; AUP 2011-145), and in the case of

TABLE 1. BASELINE POPULATION CHARACTERISTICS IN 39 DOGS WITH SPINAL CORD INJURY AND 21 HEALTHY CONTROL DOGS

Variable	SCI dogs (N=39)	Healthy control dogs (N=21)
<i>A. Continuous variables: medians (range)</i>		
Age, years	5 (1–12)	3 (1–4)
Duration of signs before admission (hours)	40.75 (2.15–169.75)	N/A
MFS at admission	3 (0–5)	N/A
TSCIS at admission	12 (0–17)	N/A
<i>B. Categorical variables: percentage (proportion)</i>		
Sex, %		
Female intact	7 (3/39)	0 (0/21)
Female spayed	44 (17/39)	14 (3/21)
Male	13 (5/39)	24 (5/21)
Male neutered	36 (14/39)	62 (13/21)
Prevalent breeds, %		
Dachshund	77 (29/39)	0 (0/21)
Labrador Retriever	0 (0/39)	33 (7/21)
Mixed breed	10 (4/39)	24 (5/21)
Level of spinal cord injury, %		
T12–T13	13 (5/39)	N/A
T13–L1	31 (12/39)	N/A
L1–L2	15 (6/39)	N/A
Other thoracic	13 (5/39)	N/A
Other lumbar	28 (11/39)	N/A
Spinal cord T2-weighted hyperintensity (available for 23 dogs that received MRI)		
Absent	70% (16/23)	N/A
Present	30% (7/23)	N/A

SCI, spinal cord injury; N/A, not applicable; MFS, modified Frankel score; TSCIS, Texas Spinal Cord Injury Score; MRI, magnetic resonance imaging.

TABLE 2. CEREBROSPINAL FLUID CYTOKINE AND CHEMOKINE CONCENTRATIONS IN 39 DOGS WITH SCI AND 21 HEALTHY CONTROL DOGS

Cytokine	SCI cases (N=39)	Control dogs (N=21)	p*	p adjusted*
GM-CSF	0.13 (0.00–19.43)	0.00 (0.00–26.65)	0.6739	0.6926
IFN- γ	0.00 (0.00–421.33)	135.12 (0.00–408.30)	0.0146	0.1460
KC-like	94.77 (0.00–604.62)	155.01 (0.00–263.49)	0.0604	0.4228
IP-10	0.00 (0.00–5.18)	1.35 (0.00–3.68)	0.0006	0.0066
IL-2	0.00 (0.00–15.08)	0.00 (0.00–16.73)	0.2822	0.6926
IL-6	0.49 (0.00–55.95)	1.50 (0.00–36.42)	0.3579	0.6926
IL-7	1.54 (0.00–22.90)	0.00 (0.00–26.36)	0.0950	0.5700
IL-8	7.35 (0.00–145.48)	0.00 (0.00–161.44)	0.0001	0.0013
IL-10	0.74 (0.00–36.45)	4.62 (0.00–47.14)	0.0365	0.2920
IL-15	0.87 (0.00–26.45)	1.06 (0.00–80.97)	0.4296	0.6926
IL-18	1.35 (0.00–45.21)	17.07 (0.00–79.8)	0.0006	0.0066
MCP-1	34.07 (0.00–3032.70)	57.13 (0.00–156.44)	0.6926	0.6926
TNF- α	0.00 (0.00–0.00)	0.00 (0.00–25.77)	0.0173	0.1557

Comparisons between injured and healthy dog cerebrospinal fluid cytokine and chemokine concentrations were made using Wilcoxon's rank-sum tests with adjustment for multiple comparisons using the method of Hochberg.

**p* is unadjusted *p* value; *p* adjusted is *p* value adjusted using the method of Hochberg.

SCI, spinal cord injury; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- γ , interferon gamma; KC, keratinocyte chemoattractant; IP-10, IFN- γ -inducible protein-10; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; TNF- α , tumor necrosis factor alpha.

client-owned dogs, were performed with written consent from each dog's owner. All studies adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

An archival bank of CSF samples housed at Texas A&M University since December 2009 and stored at -80°C was searched in March 2013 for samples from dogs with SCI resulting from IVDH. Dogs had to meet the following inclusion criteria: 1) IVDH located between the T3 and L6 vertebrae; 2) <7-day duration of neurological dysfunction; 3) treatment consisting of surgical decompression and physical rehabilitation; and 4) sufficient archival CSF available for cytokine and chemokine determination. Dogs were excluded from the study if they were enrolled in ongoing neuroprotective clinical trials, did not have 42-day postsurgical follow-up examination, or had undergone a myelogram.

Archival CSF samples from purpose-bred dogs with normal neurological examination, physical examination, complete blood cell counts, and serum biochemical analysis were used as a control population. CSF samples were obtained and stored using the same methods as described below for SCI dogs. Samples of CSF from control dogs were required to have normal total nucleated cell count (TNCC; <5 cells/ μL) and microprotein (≤ 35 mg/dL).

Animal procedures

Dogs with SCI received physical and neurological examination, complete blood count, and serum biochemistry before anesthesia. Dogs were premedicated with a combination of glycopyrrolate (Robinul-V; West-Ward Pharmaceutical Corp., Eatontown, NJ) and oxymorphone (Numorphan; Endo Pharmaceuticals, Chadds Ford, PA) or hydromorphone, induced with propofol (Rapinivet; Abbott Labs, Chicago, IL), and then intubated and maintained under general anesthesia with a combination of sevoflurane and oxygen inhalant. CSF was acquired from the cerebellomedullary cistern for routine analysis, and a 200- μL aliquot was stored at -80°C for cytokine and chemokine determination. The thoracolumbar vertebral column was imaged in dogs with SCI after CSF collection with magnetic resonance imaging (MRI) or computed tomography. The imaging technique utilized depended on the availability of equipment. A hemilaminectomy surgery was performed in all cases after imaging procedures to remove herniated disk material and any associated epidural hemorrhage; IVDH was confirmed by gross inspection or histopathology of herniated disk material. After surgery, dogs were hospitalized in an intensive care unit for 24 h, where analgesia and bladder evacuation were performed. Physical rehabilitation exercises were initiated beginning 24 h after surgery and consisted of passive range of motion, supported standing, and supported overland walking. Animals were released to owners if they could voluntarily void urine or undergo manual bladder expression and had postsurgical pain that was well controlled. Owners were instructed to continue physical rehabilitation exercises at home for 6 weeks.

Neurological scores

Dogs with SCI were neurologically examined at initial admission and at 42-day, in-hospital recheck using two ordinal SCI scores.¹⁷ In both scoring systems, dogs were considered ambulatory if they could spontaneously rise and take 10 steps without support. Postural reactions were evaluated by placing the paw on its dorsum while the dog was held in a standing position and waiting for correction. Superficial and deep nociception were tested by pinching the interdigital webbing and cross-clamping the nail bed with hemostats, respectively. Nociception was deemed to be present if there was a behavioral (e.g., vocalizing) or physiological (e.g., tachycardia) response to stimulation. Both ordinal SCI scores used in this study were previously validated in dogs with IVDH and have been shown to have excellent inter-rater agreement, correlate

to MRI signal changes within the spinal cord after injury, and predict long-term outcome.¹⁷

The modified Frankel score (MFS)¹⁷ was developed as a coarse SCI score that would permit stratification of the population into groups that parallel those used in the American Spinal Cord Injury

FIG. 1. Box-and-whiskers plots summarizing IL-8 (A), IL-18 (B), and IP-10 (C) concentrations in the cerebrospinal fluid of 39 dogs with SCI and 21 healthy control dogs. IL-8 concentration was significantly higher ($p=0.0013$) in dogs with SCI, compared to healthy dogs. IL-18 and IP-10 concentrations were both significantly lower in dogs with SCI, compared to healthy dogs ($p=0.0066$ for both). Comparisons were made using Wilcoxon's rank-sum tests with adjustments for multiple comparisons using the method of Hochberg. IL, interleukin; IP-10, interferon-gamma-inducible protein-10; SCI, spinal cord injury.

TABLE 3. CORRELATION BETWEEN CEREBROSPINAL FLUID CYTOKINE AND CHEMOKINE CONCENTRATIONS AND DURATION OF INJURY AT THE TIME OF SAMPLING IN 39 DOGS WITH SPINAL CORD INJURY

Cytokine	Coefficient (SEM)	R ²	p value Adjusted	p value
GM-CSF	-0.365 (0.133)	0.15	0.0095	0.1045
IFN- γ	-0.666 (0.331)	0.07	0.0516	0.2795
KC-like	-0.2335 (0.182)	0.02	0.2062	0.4328
IP-10	-0.174 (0.078)	0.10	0.0311	0.2177
IL-2	-0.217 (0.110)	0.06	0.0559	0.2795
IL-6	-0.282 (0.176)	0.04	0.1171	0.4328
IL-7	-0.381 (0.155)	0.12	0.0189	0.2046
IL-8	-0.566 (0.181)	0.21	0.0035	0.0420
IL-10	-0.424 (0.172)	0.12	0.0186	0.2017
IL-15	-0.379 (0.169)	0.10	0.0306	0.2177
IL-18	-0.191 (0.178)	<0.01	0.2908	0.4328
MCP-1	-0.173 (0.218)	0.01	0.4328	0.4328
TNF- α	ND	ND	ND	N/A

Data were log₁₀-transformed and analyzed using generalized linear modeling.

^aNot performed because all values of TNF- α were below the level of detection.

SEM, standard error of the mean; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- γ , interferon gamma; KC, keratinocyte chemoattractant; IP-10, IFN- γ -inducible protein-10; IL, interleukin; MCP-1, monocyte chemotactic protein 1; TNF- α , tumor necrosis factor alpha; ND, none detected; N/A, not applicable.

Association Impairment Scale (ASIA). MFS scores were as follows: 0=paraplegic with no deep nociception; 1=paraplegic with deep nociception intact; 2=paraplegic with superficial nociception intact; 3=nonambulatory paraparetic; 4=ambulatory paraparetic; and 5=paraspinal hyperesthesia as the sole clinical sign.

The Texas Spinal Cord Injury Score (TSCIS)¹⁷ was developed to be a more refined grading system than the MFS and was used for analyses that did not require stratification into large functional categories. Scores were performed in each pelvic limb. Nociception was scored as follows: 0=absent; 1=deep nociception present

only; and 2=both superficial and deep nociception present. Gait scores ranged from 0 to 6 and reflected the degree of weight support and limb protraction. Gait classifications included: 0=no voluntary motor function present when supported; 1=intact limb protraction with no ground clearance; 2=intact limb protraction with inconsistent ground clearance; 3=intact protraction with consistent ground clearance; 4=ambulatory, consistent ground clearance with moderate paresis-ataxia (will fall occasionally); 5=ambulatory, consistent ground clearance with mild paresis-ataxia (does not fall, even on slick surfaces); and 6=normal gait. Proprioceptive

FIG. 2. Scatter plot of cerebrospinal fluid concentration of IL-8 among 39 dogs with spinal cord injury, demonstrating a significant ($p=0.042$; regression coefficient = -0.0040 ; $R^2=0.144$ by generalized linear model) negative association of IL-8 concentration with duration of injury at the time of sample acquisition. IL, interleukin.

positioning was classified as: 0=absent; 1=delayed (correction occurred in > 1 sec); or 2=normal response.

Cerebrospinal fluid analysis and cytokine/chemokine determination

Approximately 1 mL of CSF was analyzed within 1 h of collection for microprotein concentration, TNCC, red blood cell (RBC) count, and cell count differential. CSF pleocytosis was defined as >5 TNCC/ μL . Normal CSF protein concentration was considered to be ≤ 35 mg/dL. Cytokine and chemokine concentrations were determined using archival CSF frozen at -80°C within 1 h of collection. CSF concentrations of IL-2, -6, -7, -8, -10, -15, and -18, granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN- γ), keratinocyte chemoattractant (KC)-like protein, IFN- γ -inducible protein-10 (IP-10), MCP-1, and tumor necrosis factor alpha (TNF- α) were measured using a Canine Cytokine Magnetic Panel kit. Assays were performed according to manufacturer instructions (Millipore Corporation, Billerica, MA) on a Bio-Rad (Hercules, CA) Bio-Plex system employing Luminex technology.

Statistical analysis

Baseline population data that were continuous were summarized using medians and ranges and categorical data were summarized using contingency tables. Cytokine and chemokine concentrations in the CSF of SCI and control dogs were compared using Wilcoxon's rank-sum tests and chi-squared tests.¹⁸ Wilcoxon's rank-sum tests, chi-squared tests, and Kruskal-Wallis' tests were used to assess relationships between CSF cytokine and chemokine concentrations and duration of SCI before CSF acquisition, SCI severity, long-term recovery as measured by TSCIS, and cytologic characteristics of CSF. Significance was set at $p < 0.05$.¹⁸ Multiple comparison adjustment was performed using the method of Hochberg.¹⁸ Correlation between continuous variables was assessed using a generalized linear model to calculate regression coefficient parameters, R^2 values, and statistical significance that the coefficient was significantly different than 0.¹⁹ When necessary, data were transformed ($\log_{10}$ function) to meet distributional assumptions of modeling. Goodness of model fit was assessed by visual inspection of diagnostic residual plots. All analyses were performed using S-PLUS statistical software (version 8.2; TIBCO).

Results

Population characteristics

There were 39 dogs in the SCI group (Table 1). The median age was 5 years (range, 1–12), and there were 3 females (7%), 17 spayed females (44%), 5 males (13%), and 14 castrated males (36%). The three most common breeds in the SCI group were: Dachshunds (29; 77%); mixed breed (4; 10%); and Shih Tzu (2; 5%). The median MFS before CSF acquisition was 3 (non-ambulatory paraparesis; range, 0–5). The median time from onset of injury to CSF collection was 40.75 hours (range, 2.15–169.75). Glucocorticoids or nonsteroidal anti-inflammatory drugs were administered to 13 (33%) and 12 (31%) dogs, respectively, with SCI before CSF acquisition.

There were 21 dogs in the control group. The median age was 3 years (range, 1–4), and there were 3 spayed females (14%), 5 males (24%), and 13 castrated males (62%). The three most common breeds were: Labrador Retriever (7; 33%); Beagle hound (5; 24%); and mixed breed (5; 24%). No control dogs received glucocorticoids or nonsteroidal drugs.

Cerebrospinal fluid analysis in dogs with spinal cord injury

The median TNCC was 2 cells/ μL (range, 0–107), the median RBC was 7 cells/ μL (range, 0–11,005), and the median microprotein was 16 mg/dL (range, 10–35; Supplementary Table 1) (see online supplementary material at <http://www.liebertpub.com>). Seven dogs had CSF pleocytosis (TNCC >5 cells/ μL). Of these dogs, the median percentage of neutrophils was 55% (range, 0–85), lymphocytes was 8% (range, 0–32), and monocytes was 25% (range, 8–100).

Cerebrospinal fluid cytokines and chemokines are dysregulated in dogs with spinal cord injury

The concentration of IL-8 was significantly ($p = 0.0013$) higher in dogs with SCI (median, 7.35 pg/mL; range, 0–145.48), compared to control dogs (median, 0; range, 0–161.44 pg/mL; Table 2; Fig. 1). However, the concentrations of IP-10 and IL-18 were

FIG. 3. Scatter plots of $\log_{10}$ -transformed cerebrospinal fluid (CSF) concentrations of KC-like protein (A) and MCP-1 (B) versus CSF microprotein concentration in 39 dogs with spinal cord injury. The concentration of CSF microprotein was significantly and positively correlated ($p = 0.0044$; regression coefficient = 1.012; $R^2 = 0.27$) with CSF KC-like protein concentration by generalized linear modeling. CSF microprotein concentration was also positively correlated with CSF MCP-1 concentration ($p < 0.0001$; regression coefficient = 1.537; $R^2 = 0.46$), using a generalized linear model. MCP-1, monocyte chemoattractant protein 1; KC, keratinocyte chemoattractant.

significantly ($p=0.0066$ for both) lower in the SCI group, compared to control dogs. No other cytokines or chemokines differed significantly between groups when adjusting for multiple comparisons. Cytokine and chemokine concentrations tended to be lower in dogs that had a longer duration of time between SCI and CSF sampling (Table 3). Only IL-8, however, was significantly and negatively ($p=0.0420$; Fig. 2) associated with duration of SCI before CSF acquisition.

Chemokines in the CSF of dogs with SCI were likewise correlated with derangements in CSF total protein concentration and RBC. The concentration of CSF total protein was significantly and positively correlated ($p=0.0044$; regression coefficient=1.012; $R^2=0.27$; Fig. 3A) with CSF KC-like protein concentration after adjusting for multiple comparisons. Additionally, CSF total protein concentration was also positively correlated with MCP-1 concentration ($p<0.0001$; regression coefficient=1.537; $R^2=0.46$; Fig. 3B) after adjusting for multiple comparisons. The CSF RBC was significantly and positively correlated with CSF MCP-1 concentration ($p<0.0001$; regression coefficient=0.292; $R^2=0.37$; Fig. 4) after adjusting for multiple comparisons. None of the other cytokines or chemokines was significantly associated with CSF total protein, CSF RBC, or CSF TNCC.

Cerebrospinal fluid cytokines and chemokines were not uniformly correlated with spinal cord injury severity or recovery

There were no significant differences in the concentrations of cytokines or chemokines in CSF among dogs categorized by their SCI severity score (MFS) at the time of CSF sampling (Supplementary Table 2) (see online supplementary material at <http://www.liebertpub.com>). Only MCP-1 concentration was signifi-

cantly, negatively associated ($p<0.0001$; regression coefficient=−75 [95% confidence interval, −101 to −49]; $R^2=0.36$; Fig. 5) with the 42-day outcome, as measured by an ordinal motor, postural, and sensory score (TSCIS).

Discussion

This study represents the first broad assessment of inflammatory cytokine and chemokine profiles in the CSF of dogs with naturally occurring acute SCI. Here, we demonstrated that CSF IL-8 was elevated after injury and was negatively correlated with duration of SCI before sampling. Two proinflammatory molecules (IP-10 and IL-18) had a significantly lower concentration in the CSF of injured dogs, compared to healthy control animals. Total protein concentration in the CSF, a marker of blood–spinal cord barrier (BSCB) disruption, was positively correlated with CSF MCP-1 and KC-like protein concentrations. Further, CSF MCP-1 concentration was negatively associated with behavioral measures of long-term outcome after SCI.

Data from this study identified IL-8 as an early mediator of inflammation in dogs with thoracolumbar IVDH-associated SCI. In humans, expression of CSF or spinal cord IL-8 peaks within 24 h of SCI, likely arises from a variety of local sources, including activated microglia, and has been associated with neutrophilic infiltration into parenchyma.^{16,20} In the population studied here, increases in CSF nucleated cell count were uncommonly noted (18%; 7 of 39 dogs), but, when present, had a substantial neutrophilic component. In a previous study that examined mRNA expression of cytokines in the spinal cord of dogs with IVDH-associated SCI, IL-8 was likewise found to be increased, compared to control dogs, especially in acute injury.⁹ It should be noted that neutrophilic inflammation was not prevalent in animals in that study, which is in contrast to most other reports that have examined spinal cord or

FIG. 4. Scatter plot of $\log_{10}$ -transformed cerebrospinal fluid (CSF) MCP-1 concentration versus CSF red blood cell (RBC) concentration in 39 dogs with spinal cord injury. The CSF RBC was significantly and positively correlated with CSF MCP-1 concentration ($p<0.0001$; regression coefficient=0.292; $R^2=0.37$) using generalized linear modeling. MCP-1, monocyte chemotactic protein 1.

FIG. 5. Scatter plot of $\log_{10}$ -transformed values of MCP-1 concentrations in the cerebrospinal fluid (CSF) versus TSCIS (a validated behavioral measure of spinal cord injury severity) on day 42 postinjury in 39 dogs with intervertebral disk herniation. There was a significant negative association between CSF MCP-1 concentration at admission and TSCIS on day 42 ($p < 0.0001$; $R^2 = 0.36$) using generalized linear modeling. The slope of the solid line is the regression coefficient estimated from the generalized linear model, and dotted lines are the 95% confidence intervals. TSCIS, The Texas Spinal Cord Injury Score; MCP-1, monocyte chemoattractant protein 1.

CSF samples from dogs with IVDH-associated SCI and tissue from other species with acute injury.^{21–24}

We did not find that IL-6 was increased after SCI and identified down-regulation of two proinflammatory mediators in injured dogs. In humans with SCI, IL-6 concentration is increased in the CSF, compared to healthy individuals, and peaks approximately 24 h postinjury.¹³ In the spinal cord of dogs with IVDH, IL-6 mRNA has been shown to be increased after acute SCI and IL-6 protein has been found to be overexpressed in rodent contusion models.^{9,20,25} IL-18 and IP-10 have not been previously examined as mediators of injury in dogs with SCI and were decreased in affected animals in this study, compared to control animals. In rodent models of injury, IP-10 and IL-18 has been shown to be increased after SCI, and in humans, CSF IP-10 was increased beyond reference concentrations.^{13,26,27} The reasons for a lack of IL-6 dysregulation in dogs reported here and the decreased CSF IL-18 and IP-10 are likely numerous. First, only a small number of dogs studied here were severely injured (loss of limb movement and nociception), in contrast to the previously reported dogs (all were euthanized because of severe injury) and humans. Second, detection of cytokines and chemokines in the CSF may be challenging because of failure of these substances to elute from parenchyma.^{28,29} Third, a majority of affected dogs in this study were treated with either glucocorticoids or nonsteroidal drugs before CSF acquisition, which can modulate immune responses.³⁰ Though previously reported dogs and humans were also treated with these agents, the type of drug, dose, and duration of treatment could have varied. Finally, and perhaps most critically, because the mean time between SCI and CSF acquisition was 40 h in this study, cytokines

and chemokines that peak within just a few hours of injury might not have been detectable in the majority of animals here. In rodents with experimental SCI, peak CSF IL-6 concentration occurs only 6 h after injury and samples obtained 24 h after SCI had IL-6 concentration that was not significantly different than sham-treated animals.²⁰

We assessed relationships between CSF analytes and cytokine/chemokine concentrations in dogs with SCI to better understand pathologic processes associated with cytokine and chemokine up-regulation. Both MCP-1 and KC-like protein concentration in the CSF were positively correlated with CSF microprotein concentration, and MCP-1 was positively correlated with RBC concentration. The sources of MCP-1 and KC-like protein likely include the endothelium, which becomes disrupted during SCI and can facilitate the entry of peripheral macrophages, another source of both of these chemokines.²⁷ It is therefore expected that indicators of BSCB disruption, such as CSF RBC and microprotein, might correlate with increases in these chemokines. Whereas KC-like protein has not been broadly examined in SCI, in spinal cord-injured humans, CSF MCP-1 is increased above reference ranges.¹³ Concentrations were highest early after SCI, when CSF RBC concentration was highest. Similar increases in spinal cord tissue MCP-1 protein and mRNA have been reported in rodents with experimental spinal cord contusion.^{20,27}

CSF MCP-1 concentration was associated with neurological outcome at 42 days post-SCI, as measured by a validated ordinal score. However, no other cytokines or chemokines were associated with either initial SCI severity or 42-day post-SCI ordinal injury score. In humans with SCI, the only CSF cytokine or chemokine

associated with SCI severity is IL-8, which is also predictive of ASIA status 6 months after admission.¹³ Interspecies differences in neuroinflammation after SCI, the small number of severely impaired dogs and dogs with poor long-term outcome in this study, differences in timing of CSF acquisition, the use of surviving patients that did not have spinal cord tissue analyzed, and other factors related to treatment before and after CSF sampling may explain the different results in humans and dogs in this report.

Classically, inflammatory responses after SCI have been evaluated histologically using rodent contusion models. Data acquired through this approach have been a foundation for pre-clinical neuroprotective studies and early-phase human clinical trials.^{13,30,31} Here, we utilized CSF to assess cytokine and chemokine profiles in a naturally occurring, clinically relevant, large-animal form of compressive/contusive SCI. There are limitations inherent to using naturally occurring disease in which many animals survive SCI, including variation in anatomical level of injury, pretreatment with drugs that can modulate immune responses, and lack of available histological samples. Nonetheless, our results largely confirm the involvement of IL-8 and MCP-1 in SCI pathogenesis and highlight the possibility of targeting these molecules in canine pre-clinical trials.

Conclusion

CSF cytokines and chemokines involved in innate inflammatory responses are dysregulated after naturally occurring, acute thoracolumbar SCI in dogs. CSF IL-8 was elevated and correlated to duration of SCI before sampling, as has been observed in humans with SCI, but an association between measurements of neurological recovery and CSF IL-8 was not detected. CSF MCP-1, which is elevated in humans with SCI, was positively correlated to CSF microprotein and RBC concentrations and was associated with neurological recovery at day 42 after SCI. These data would suggest that neuroinflammatory processes in humans and dogs with SCI do share certain broad parallels. The mechanisms underlying the decreased CSF IP-10 and IL-18 concentrations in SCI dogs, compared to controls, are unclear and merit further evaluation.

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Author Disclosure Statement

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