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Cytokine Concentrations in the Cerebrospinal Fluid of Great Danes with Cervical Spondylomyelopathy

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Background: Chronic inflammation is involved in the pathogenesis of human cervical spondylotic myelopathy and could also play a role in cervical spondylomyelopathy (CSM) in dogs.

Hypothesis/Objectives: That cerebrospinal fluid (CSF) cytokine concentrations would differ between clinically normal (control) and CSM-affected Great Danes (GDs), with affected GDs showing higher levels of inflammatory cytokines, such as interleukin (IL)-6 and monocyte chemoattractant protein-1/chemokine ligand 2 (MCP-1/CCL2).

Animals: Client-owned GDs: 15 control, 15 CSM-affected.

Methods: Prospective study. Dogs underwent cervical vertebral column magnetic resonance imaging and collection of CSF from the cerebellomedullary cistern. Cytokine concentrations were measured using a commercially available canine multiplex immunoassay. Cytokine concentrations were compared between groups. Associations with the administration of anti-inflammatory medications, disease duration and severity, severity of spinal cord (SC) compression, and SC signal changes were investigated in affected GDs.

Results: Affected GDs had significantly lower MCP-1/CCL2 (mean 138.03 pg/mL, 95% confidence interval [CI] = 114.85–161.20) than control GDs (212.89 pg/mL, 95% CI = 165.68–260.11, $P = .028$). In affected GDs, MCP-1/CCL2 concentrations correlated inversely with the severity of SC compression. There were no associations with administration of anti-inflammatory medications, disease duration, or disease severity. IL-6 concentrations were significantly higher (2.20 pg/mL, 95% CI = 1.92–2.47, $P < .001$) in GDs with SC signal changes.

Conclusions and Clinical Importance: Lower MCP-1/CCL2 in CSM-affected GDs might compromise clearance of axonal and myelin debris, delay axon regeneration, and affect recovery. Higher IL-6 in CSM-affected GDs with SC signal changes suggests more severe inflammation in this group.

Key words: Biomarker; Dog; Spinal cord disease; Wobbler syndrome.

Osseous-associated cervical spondylomyelopathy (CSM) frequently affects Great Danes (GDs).[a,1,2] CSM in dogs shares similarities with cervical spondylotic myelopathy, the most common cause of chronic compressive cervical myelopathy in people.[2–4] While surgical treatment can attenuate the progression of this human disease, many patients are left with substantial neurologic disability.[3] Gaps in the knowledge of the disease pathobiology have historically limited therapeutic advances in this human condition and current studies are actively investigating the disease pathogenesis with the objective of developing new neuroprotective approaches to improve outcome.[3–9] Rodent models of cervical spondylotic myelopathy show that neuronal and oligodendrocyte apoptosis, chronic neuroinflammation, and microvascular compromise result in progressive neural degeneration and potentially irreversible spinal cord (SC) damage and could be related to treatment failure, poor long-term outcome, or both.[3–9] Similar changes have been observed in the SC of CSM-affected dogs.[b] Much like the human condition, treatment of osseous-associated CSM in dogs also yields variable results. Recurrence of clinical signs is common, and clinical deterioration can be seen months to years after treatment.[a,c,2,10,11] The molecular and biochemical mechanisms underlying CSM are poorly understood, but progress in this area is vital for development of targeted therapeutics to improve long-term outcome in dogs with CSM.[d]

The close proximity of the cerebrospinal fluid (CSF) to the SC provides a unique opportunity to assess in

Abbreviations:

CBC	complete blood count
CI	confidence interval
CSF	cerebrospinal fluid
CSM	cervical spondylomyelopathy
CXCL10	inducible protein-10
CXCL1-like	keratinocyte-derived chemokine
GDs	Great Danes
GM-CSF	granulocyte-macrophage colony-stimulating factor
IFN-γ	interferon-gamma
IL	interleukin
MCP-1/CCL2	monocyte chemoattractant protein-1/chemokine ligand 2
MRI	magnetic resonance imaging
RBC	red blood cell
RR	reference range
SC	spinal cord
T2-WI	T2-weighted images
TE	time to echo
TNCC	total nucleated cell count
TNF-α	tumor necrosis factor-α
TR	repetition time

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vivo the presence of possible biomarkers in neurologic disease.[12–14] A recent study showed that the blood–SC barrier is disrupted with chronic SC compression in a novel rat model of cervical spondylotic myelopathy.[3] Loss of the blood–SC barrier integrity can facilitate for circulating immune system cells to reach the SC.[15] Concentrations of the proinflammatory interleukins (IL)-6 and IL-8 were elevated in affected humans with cervical spondylotic myelopathy when compared to controls.[16,17] No studies have investigated the CSF cytokine profile of dogs with CSM.

The goal of this study was to prospectively compare the CSF cytokine concentrations of GDs with and without clinical signs of CSM. We also investigated associations between the cytokine concentrations and the administration of anti-inflammatory medications, disease duration, severity of clinical signs, severity of SC compression, and presence of SC signal changes on magnetic resonance imaging (MRI) in CSM-affected GDs. We hypothesized that the CSF cytokine profile would differ between clinically normal and CSM-affected GDs, with affected GDs showing higher levels of neuroinflammatory cytokines, such as IL-6 and monocyte chemoattractant protein-1/chemokine ligand 2 (MCP-1/CCL2).

Materials and Methods

Animals

Two groups of client-owned GDs were prospectively enrolled between April 2011 and October 2012. The investigation was conducted in accordance with the guidelines and with approval of The Ohio State University Clinical Research Advisory Committee and the Institutional Animal Care and Use Committee. Written owner consent was obtained before study enrollment.

The 1st group included 15 skeletally mature GDs (>1 year of age) defined as clinically normal (control) based on a normal neurologic examination and no history of neurologic disease. The 2nd group included 15 GDs with clinical signs and neurologic examination findings consistent with CSM and confirmation via MRI. A video of the gait of all CSM-affected dogs was obtained at the time of enrollment. The duration of clinical signs and the administration of any medications at the time of enrollment were recorded. All 30 GDs were examined by 2 investigators (PMV and RdC), and underwent complete blood counts (CBC), serum biochemistry profiles, MRI of the cervical vertebral column and SC, and CSF analysis.

Gait Grading

Videos were reviewed at a later time by 1 investigator (PMV) and used to assign a neurologic grade to each CSM-affected GD. At least 2 minutes of video material was available for all affected dogs. Gait was graded for each thoracic and pelvic limb as follows: grade 0, normal limb; grade 1, abnormal use of the limb <40% of the steps; grade 2, abnormal use of the limb between 40 and 70% of the steps; and grade 3, abnormal use of the limb >70% of the steps. Signs of both paresis (ie, knuckling, scuffing, dragging) and general proprioceptive ataxia (inconsistent limb/foot placement) were considered as an abnormal use of the limb. If the grade assigned to the right and left thoracic limbs differed, the worse grade (from 0 to 3) was used as the overall grading for that pair of limbs. The same process was followed up for the pelvic limb gait grading. The thoracic limb grade (from 0 to 3) and the pelvic limb grade (from 0 to 3) were summed for each dog, producing an overall final gait grade ranging from 1 to 6 (no CSM-affected dogs had all 4 limbs characterized as normal, thus no overall final grade of 0 was possible). For statistical associations between severity of clinical signs and CSF cytokine concentrations, dogs with gait grades of 1 and 2 were categorized as mild, those with grades of 3 and 4 were considered moderate, and those with grades of 5 and 6 were categorized as severe.

MRI Protocol and Image Evaluation

Magnetic resonance imaging of the cervical vertebral column was obtained under general anesthesia with a 3.0 Tesla magnet[e] and a surface coil. Dogs were positioned in dorsal recumbency with the head and neck in neutral position. Turbo-spin echo sagittal and transverse T2-weighted images (WI) were obtained. Repetition time (TR) and time to echo (TE) were as follows: sagittal T2-WI, TR = 5,000 milliseconds, TE = 110 milliseconds; transverse T2-WI, TR = 4,000 milliseconds, TE = 120 milliseconds. The field of view was 30 cm in the sagittal plane and 20 cm in the transverse plane. Slice thickness was set at 3 mm with no interslice interval. Seven intervertebral spaces (C2-3 to T1-2) were imaged in all dogs. Five transverse slices were obtained for every intervertebral space. The transverse slices were aligned parallel to the intervertebral disk and arranged to pass through the center of each intervertebral space as well as the cranial and caudal end plates of the adjacent vertebral bodies.

All MRI studies were evaluated by 1 investigator (PMV) by dedicated software.[f] All of the sagittal and transverse images available for every intervertebral space were subjectively evaluated to determine the sites of SC compression and presence of SC signal changes. SC compression was graded as previously described:[18] mild (<25% reduction in the SC diameter), moderate (25–50% reduction), and severe (>50% reduction in the SC diameter). If more than 1 grade of severity of SC compression was present in the same dog, the most severe grade was used for statistical analysis. The sites of SC T2 hyperintensity were recorded and affected dogs classified accordingly as either having or not having SC signal changes.

CSF Collection and Analyses

Cerebellomedullary cistern CSF samples were collected under general anesthesia within 5 minutes after performing the cervical vertebral column MRI. All CSF samples were obtained by 1 of 2 investigators (PMV and RdC) using spinal 1.5 in, 21G needles into sterile glass red top tubes. A volume of 3–5 mL of CSF divided into 2–3 tubes was obtained for each dog. Within 30 minutes of collection, 1 mL of CSF was used to obtain the total nucleated cell count (TNCC), red blood cell (RBC) count, cytocentrifuge slide and differential cell count, and protein concentration at the Veterinary Medical Center, The Ohio State University clinical pathology laboratory. Protein was measured by benzethonium chloride precipitation with a lower limit of detection of 4 mg/dL.[g] The laboratory reference range (RR) for cerebellomedullary cistern CSF protein is <25 mg/dL. The TNCC and RBC count were determined manually in undiluted CSF with a hemocytometer. The RR for both TNCC and RBC count is ≤5 cells/μL. Slides for the differential cell count were prepared by cytocentrifugation[h] of 120 μL for 5 minutes at 350 × g with subsequent Wright's Giemsa staining. Cell distribution was considered normal if it contained 60–70% macrophages, 30–40% lymphocytes, and 0% neutrophils.[19]

A volume of 2 mL of CSF from each dog was immediately centrifuged at 1,100 × g for 8 minutes to remove cellular materials,

and the supernatants were collected and stored at −80°C until further analysis. A commercially available canine-specific multiplex immunoassay[i] was used following manufacturer's instructions to quantify the following cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ), inducible protein 10 (CXCL10), IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, keratinocyte-derived cytokine (CXCL1-like), MCP-1/CCL2, and tumor necrosis factor-α (TNF-α). The cytokine laboratory assays were performed at The Dorothy M. Davis, Heart & Lung Research Institute, The Ohio State University Medical Center. The assay was performed according to manufacturer's direction with overnight incubation of the plate on a plate shaker at 4°C. The plate was read on a Luminex 200 instrument[j] and the resulting data were evaluated by dedicated software (STarStation Software version 2.3; Luminex).[j]

All standard, quality controls, and samples were analyzed in duplicates. The limits of detection for the cytokines analyzed were: GM-CSF (9.2 pg/mL), IFN-γ (13.6 pg/mL), CXCL10 (3.2 pg/mL), IL-2 (3.5 pg/mL), IL-6 (3.7 pg/mL), IL-7 (7.5 pg/mL), IL-8 (21.7 pg/mL), IL-10 (8.5 pg/mL), IL-15 (9.0 pg/mL), IL-18 (5.8 pg/mL), CXCL1-like (5.3 pg/mL), MCP-1/CCL2 (21.0 pg/mL), and TNF-α (6.1 pg/mL).

Statistical Analysis

Protein, TNCC, RBC count, and cytokine concentrations were compared between control and CSM-affected GDs by use of linear regression analysis adjusted for age, sex, weight, and administration of anti-inflammatory medication by commercially available software.[k] Linear regression analysis adjusted for age, sex, and weight was also used to test associations between protein, TNCC, RBC count, and cytokine concentrations, and the use of anti-inflammatory medications, severity of clinical signs, severity of SC compression, and SC signal changes in the CSM-affected GDs. The Holm's procedure was used to adjust the *P* values to conserve the overall type I error at .05. A Spearman's rank correlation test was used to investigate if any associations existed between the duration of clinical signs and the CSF parameters (protein, TNCC, RBC count, and cytokine concentrations). Spearman's correlation coefficient (rho) values close to 1 indicate a perfect positive linear correlation, values close to −1 indicate a perfect negative linear correlation, and values close to 0 indicate no linear correlation. Significance was set at a *P* value <.05.

Results

Clinical Data and Gait Grading

Clinically normal GDs included 7 females (6 spayed, 1 intact) and 8 males (7 neutered, 1 intact). Their median age at the time of study enrollment was 2.3 years (range, 1–6.4 years). The median weight was 52 kg (range, 40.5–73 kg). All control GDs had normal neurologic examination and CBC and serum biochemistry, and were not receiving any medication at the time of enrollment. The CSM-affected GDs included 2 spayed females, 12 neutered males, and 1 intact male. Their median age at the time of study enrollment was 4 years (range, 1–7.2 years). Their median weight was 56.8 kg (range, 42–79.3 kg). The reported median age at the onset of signs was 1.7 years (range, 0.4–4.2 years). The clinical signs had been present for a mean time of 1.9 years (range, 0–5 years) before study enrollment. Fourteen of the 15 CSM-affected dogs showed ambulatory tetraparesis with general proprioceptive ataxia of all 4 limbs. One CSM-affected dog showed a spastic thoracic limb gait with ambulatory paraparesis and general proprioceptive ataxia of the pelvic limbs. All CSM-affected GDs had delayed postural reactions involving all 4 limbs and mild neck pain was elicited in 6. The summed gait grading for the thoracic and pelvic limbs yielded the following results: grade 1, n = 1 dog; grade 2, n = 3; grade 3, n = 1; grade 4, n = 3; grade 5, n = 1; grade 6, n = 6. For statistical analysis, 4 dogs were considered to have mild signs (grades 1 and 2), 4 had moderate signs (grades 3 and 4), and 7 had severe signs (grades 5 and 6). Eleven of the 15 CSM-affected GDs were receiving anti-inflammatory medication at the time of enrollment. Seven CSM-affected GDs were receiving prednisone (dose range from 0.34 mg/kg/every third day to 0.6 mg/kg/day), 1 was receiving dexamethasone (0.064 mg/kg/day), 2 were receiving carprofen[l] (2.3 mg/kg/twice a day), and 1 received meloxicam[m] (0.11 mg/kg/day). The bloodwork values outside of the RR in the CSM-affected GDs were considered consistent with the administration of anti-inflammatory medications.

MRI Evaluation

One of the control GDs had 2 sites of SC compression at C4-5 and C5-6. Forty-four sites of SC compression were identified in the 15 CSM-affected GDs. Based on the severity of the SC compression recorded on MRI, 2 dogs were classified as having mild compression, 3 had moderate SC compression, and 10 had severe compression. Five dogs had SC compression at 4 separate sites, 6 dogs had SC compression at 3 sites, 2 dogs had SC compression at 2 sites, and an additional 2 dogs had 1 site each of SC compression. The sites affected by SC compression in CSM-affected GDs (in decreasing order) included C4-5 and C6-7 (12 dogs each), C5-6 (10 dogs), C2-3 (5 dogs), C3-4 (3 dogs), and C7-T1 (2 dogs). No SC signal changes were recorded in the control GDs. Nine of the 15 CSM-affected GDs had sites of SC T2 hyperintensity. One dog had 3 sites of SC hyperintensity, 3 dogs had 2 sites each, and 5 dogs had 1 site each. The hyperintense SC signal changes were recorded at sites of moderate or severe SC compression in all affected dogs.

Cerebrospinal Fluid Results

No significant differences between control and CSM-affected GDs were identified for CSF protein, TNCC, or RBC count (Table 1). No differential cell count abnormalities were identified on any dog. Table 1 also compares the data for the measurable CSF cytokine concentrations between both groups. A significant difference between control and affected GDs was recorded for MCP-1/CCL2. The concentrations of GM-CSF, IFN-γ, CXCL10, IL-2, IL-7, IL-8, IL-15, IL-18, and TNF-α were below the detection limits.

No significant differences in the concentrations of protein (*P* = .67), TNCC (*P* = .94), RBC count (*P* = .84), IL-6 (*P* = .19), CXCL1-like (*P* = .31), IL-10

Table 1. Comparison of CSF protein, RBC count, TNNC, and CSF cytokines (IL-6, CXCL1-like, IL-10, and MCP-1/CCL2) in control GDs and GDs with CSM.

CSF Parameter	Units	Control	CSM-affected	*P* value[a]
Protein	mg/dL	14.4 (11.3, 17.6)	12.4 (10.9, 13.8)	.74
RBC	cells/μL	4.07 (0.40, 7.74)	1.36 (−0.24, 2.97)	.83
TNCC	cells/μL	1.34 (−0.92, 3.60)	0.80 (−0.25, 1.85)	.67
IL-6	pg/mL	1.96 (0.51, 3.41)	1.63 (0.88, 2.38)	.670
CXCL1-like	pg/mL	104.76 (13.25, 196.27)	208.42 (165.99, 250.85)	.22
IL-10	pg/mL	6.02 (3.16, 8.89)	4.38 (3.12, 5.64)	.65
MCP-1/CCL2	pg/mL	212.89 (165.68, 260.11)	138.03 (114.85, 161.20)	.028[b]

CSF, cerebrospinal fluid; RBC, red blood cell; TNNC, total nucleated cell count; IL, interleukin; CXCL1-like, keratinocyte-derived cytokine; MCP-1/CCL2, monocyte chemoattractant protein-1; GDs, Great Danes; CSM, cervical spondylomyelopathy.

Data are presented as mean (95% confidence interval).

[a]*P* value based on a linear regression model adjusted for age, sex, weight, and administration of anti-inflammatory medication. *P* value adjusted by the Holm's procedure to preserve the type I error at .05.

[b]Indicates statistical significance, $P < .05$.

Table 2. Correlation coefficient (rho) results obtained from a Spearman's rank test to investigate possible correlations between the duration of clinical signs and the CSF parameters in 15 CSM-affected GDs.

CSF Parameter	Units	rho[a]	*P* value[b]
Protein	mg/dL	0.113	.69
RBC	cells/μL	0.039	.89
TNCC	cells/μL	0.055	.85
IL-6	pg/mL	−0.143	.74
CXCL1-like	pg/mL	0.186	.51
IL-10	pg/mL	0.187	.52
MCP-1/CCL2	pg/mL	0.418	.16

CSF, cerebrospinal fluid; RBC, red blood cell; TNNC, total nucleated cell count; IL, interleukin; CXCL1-like, keratinocyte-derived cytokine; MCP-1/CCL2, monocyte chemoattractant protein-1; GDs, Great Danes; CSM, cervical spondylomyelopathy.

[a]Correlation coefficient (rho) values close to 1 indicate a perfect positive linear correlation, values close to −1 indicate a perfect negative linear correlation, and values close to 0 indicate that there is no linear correlation.

[b]Indicates statistical significance, $P < .05$.

($P = .17$), and MCP-1/CCL2 ($P = .56$) were identified between CSM-affected GDs on no medication and those receiving corticosteroids. Similarly, no differences were identified between CSM-affected GDs on no medication and those receiving nonsteroidal anti-inflammatories for protein ($P = .53$), TNCC ($P = .80$), RBC count ($P = .46$), IL-6 ($P = .95$), CXCL1-like ($P = .49$), IL-10 ($P = .16$), or MCP-1/CCL2 ($P = .10$).

There was no correlation between the duration of the clinical signs and any of the CSF parameters evaluated in the CSM-affected GDs (Table 2). There were no statistically significant associations between CSF cytokines and severity of clinical signs in the CSM-affected GDs (Table 3). Similarly, no significant differences were identified when correlating the degree of SC compression recorded on MRI and CSF protein ($P > .99$), TNCC ($P > .99$), RBC count ($P > .99$), IL-6 ($P > .99$), CXCL1-like ($P > .99$), or IL-10 ($P > .99$) in CSM-affected GDs. However, MCP-1/CCL2 concentrations were significantly lower in CSM-affected GDs with severe SC compression (mean 119.7 pg/mL, 95% confidence interval [CI] = 98.4–141.0, $P = .002$) and moderate SC compression (mean 130.6 pg/mL, 95% CI = 91.8–169.3, $P = .022$) when compared to those that had mild SC compression (mean 254.9 pg/mL, 95% CI = 190.8–319.1). No significant difference in the MCP-1/CCL2 concentration was identified when affected GDs with moderate SC compression were compared to those with severe compression ($P > .99$). When comparing CSF data between CSM-affected GDs with and without SC signal changes, no significant differences were identified for protein ($P > .99$), TNCC ($P = .77$), RBC ($P > .99$), CXCL1-like ($P > .99$), IL-10 ($P > .99$), or MCP-1/CCL2 ($P > .99$). However, a significantly higher concentration of IL-6 ($P < .001$) was identified in CSM-affected GDs with SC signal changes (mean 2.20 pg/mL, 95% CI = 1.92–2.47) when compared to CSM-affected GDs that did not have SC hyperintensity (mean 1.00 pg/mL, 95% CI = 0.73–1.28).

Discussion

The concentration of MCP-1/CCL2 in CSF from CSM-affected GDs was significantly lower than control GDs, and correlated inversely with the severity of SC compression as determined by MRI. Also, CSM-affected GDs that had SC signal changes on MRI had significantly higher CSF concentrations of IL-6 than affected GDs with no SC signal changes.

The cytokine MCP-1/CCL2 mediates chemotaxis of monocytes to the injured nervous system.[20,21] Monocytes are involved in the inflammatory process and degradation of axons and myelin debris in nervous system injury.[20,22,23] The clearance of myelin after injury appears important for axon regeneration in the central nervous system because of the existence of axon growth inhibitors in myelin.[23,24] The MCP-1/CCL2 concentration was significantly lower in CSM-affected GDs when compared to control GDs. Within the CSM-affected GDs, MCP-1/CCL2 concentrations were lowest in those with severe and moderate SC compression versus dogs with mild compression. Lower MCP-1/CCL2

Table 3. Associations between cytokine concentrations and severity of clinical signs in 15 CSM-affected GDs. Data for each disease category (mild, moderate, and severe) are presented as mean (95% confidence interval). When comparing each cytokine between 2 categories of severity (ie, mild versus moderate), the *P* value obtained for that comparison is shown.

Severity of signs	IL-6	CXCL1-like	IL-10	MCP-1/CCL2
Mild (n = 4)[a]	1.44 (−0.22, 3.10)	210.5 (139.8, 281.2)	5.92 (3.00, 8.85)	154.7 (101.8, 207.6)
Moderate (n = 4)[a]	−0.44 (−3.55, 2.66)	195.2 (121.7, 268.7)	2.02 (−1.39, 5.42)	137.8 (68.9, 206.8)
Severe (n = 7)[a]	2.23 (0.77, 3.69)	214.7 (162.0, 267.4)	4.39 (2.19, 6.59)	115.2 (72.4, 158.0)
Mild versus moderate[b]	.29	.78	.09	.72
Mild versus severe[b]	.50	.93	.42	.25
Moderate versus severe[b]	.14	.68	.26	.60

IL, interleukin; CXCL1-like, keratinocyte-derived cytokine; MCP-1/CCL2, monocyte chemoattractant protein-1; GDs, Great Danes; CSM, cervical spondylomyelopathy.

[a]n = number of CSM-affected dogs categorized as having mild, moderate, and severe clinical signs.

[b]*P* values based on a linear regression model adjusted for age, sex, and weight. Statistical significance was set at $P < .05$.

concentrations might compromise the clearance of axonal and myelin debris in CSM-affected GDs with more severe SC compression possibly affecting recovery. A potential explanation for the lower MCP-1/CCL2 concentrations in GDs with more severe compression could be an exhausted MCP-1/CCL2 response. During central nervous system inflammation, MCP-1/CCL2 expression can be transitory.[21] In vitro studies have shown that after transcription of MCP-1/CCL2, a refractory state can ensue, during which no additional MCP-1/CCL2 can be expressed.[21] Lower MCP-1/CCL2 concentration in CSM-affected GDs might represent an exhausted response of this cytokine caused by the presence of long-standing inflammation and chronic stimulation of MCP-1/CCL2. Further histopathologic and immunohistochemical studies are needed to clarify the role of MCP-1/CCL2 in CSM in dogs.

We documented significantly higher CSF IL-6 concentrations in affected GDs that had SC T2 hyperintensities. In people, SC T2 hyperintensity can reflect a wide range of histopathologic changes, from reversible edema to irreversible necrosis.[25] IL-6 has various functions, including a role in the generation and propagation of chronic inflammation.[26] Most studies have investigated IL-6 in the setting of inflammatory SC disease or acute SC injury, and much less is known about its role in chronic compressive myelopathies.[26–28] CSF IL-6 was increased in people with cervical compressive myelopathy when compared to control individuals, but no correlation with disease severity or duration was found.[17] No studies have correlated CSF IL-6 concentrations and SC signal changes. The SC parenchyma appears more sensitive than the brain tissue to the deleterious effects of IL-6, with oligodendrocytes and axons being the primary targets of IL-6 mediated cytotoxicity.[27] It is possible that higher IL-6 concentrations in CSM-affected GDs with SC signal changes represent the presence of more severe tissue injury secondary to chronic inflammation compared to the affected GDs that do not show SC signal changes. Additional studies are warranted to investigate this finding.

A limitation of this study is that 11/15 CSM-affected GDs were receiving anti-inflammatory medications at the time of enrollment and CSF collection, making an assessment of the effects of medications on CSF cytokines difficult. In veterinary medicine, many dogs with myelopathies have already been treated with anti-inflammatories before they are referred to specialty practices and a definitive diagnosis is obtained.[29] Both corticosteroids and NSAIDs can alter the production of cytokines in vitro and in vivo.[30,31] This fact could have hindered the identification of differences between groups. However, no significant differences were identified in the cytokine concentrations between the affected GDs on anti-inflammatories and those that were not receiving medication. The lack of differences between affected GDs receiving medication and those that were not could also be a reflection of the low number of dogs in each one of these subgroups, which would have affected the power of this part of the analysis. This suggests that at least for those cytokines that were measurable, the administration of anti-inflammatories did not have a major impact on cytokine concentrations. Another limitation is that many of the cytokines were below the detection limits for both control and CSM-affected GDs, regardless of the latter being on medication or not. Human studies evaluating CSF cytokines concentrations in cervical myelopathy also found various cytokines, such as TNF-α and IL-10, that were below the detection limits.[16,17] However, that a cytokine is not measurable on CSF does not completely exclude a role in the disease pathogenesis. While TNF-α was below detectable limits in the CSF of people with cervical spondylotic myelopathy, rodent models of this human condition have shown that this cytokine contributes to oligodendrocyte apoptosis in the chronically compressed SC.[8,9] Similarly, while some of the cytokines that were below the detection limits might not be involved in the pathogenesis of CSM in dogs, others might still play a role in the disease pathogenesis but their CSF concentrations might be too low for the available techniques to detect. It is also possible that cytokine concentrations might be influenced by acute exacerbations of a chronic compression. Additional limitations are the lack of histopathologic or immunohistochemical studies to corroborate the CSF findings, and

the lack of comparison with CSF from dogs with other chronic SC diseases.

Lower MCP-1/CCL2 CSF concentrations in CSM-affected GDs might compromise the axonal and myelin debris clearance performed by monocytes, delay axon regeneration and remyelination, and affect recovery in CSM-affected GDs. Higher IL-6 CSF concentration in CSM-affected GDs with SC signal changes is suggestive of more severe neuroinflammation in this subset of affected dogs. Future research should aim at investigating the postmortem presence of these cytokines in the SC tissue of CSM-affected dogs and correlate them with the presence of SC axonal loss, demyelination, or inflammation, to further characterize the specificity of the CSF findings reported here.

Footnotes

[a] Delamaide Gasper J, Rylander H, Waller K. Joint-associated cervical spondylomyelopathy: 27 cases (2000–2012). J Vet Int Med 2013;27:676 (abstract)

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[e] Achieva 3.0 Tesla; Philips Healthcare, Best, The Netherlands

[f] E-Film Merge Healthcare, Milwaukee, WI

[g] Roche Cobas 501 analyzer; Roche Diagnostics, Indianapolis, IN

[h] Shandon Cytospin3; Life Sciences International (Europe) Limited, Astmoor, UK

[i] Milliplex MAP canine cytokine/chemokine magnetic bead panel kit, Millipore, Billerica, MA

[j] Luminex Corporation, Austin, TX

[k] Stata, version 12.1; Stata Corporation, College Station, TX

[l] Rimadyl; Pfizer Animal Health, Exton, PA

[m] Metacam; Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO

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