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Assessment of CCL2 and CXCL8 chemokines in serum, bronchoalveolar lavage fluid and lung tissue samples from dogs affected with canine idiopathic pulmonary fibrosis

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ABSTRACT

Canine idiopathic pulmonary fibrosis (CIPF) is a progressive disease of the lung parenchyma that is more prevalent in dogs of the West Highland white terrier (WHWT) breed. Since the chemokines (C-C motif) ligand 2 (CCL2) and (C-X-C motif) ligand 8 (CXCL8) have been implicated in pulmonary fibrosis in humans, the aim of the present study was to investigate whether these same chemokines are involved in the pathogenesis of CIPF. CCL2 and CXCL8 concentrations were measured by ELISA in serum and bronchoalveolar lavage fluid (BALF) from healthy dogs and WHWTs affected with CIPF. Expression of the genes encoding CCL2 and CXCL8 and their respective receptors, namely (C-C motif) receptor 2 (CCR2) and (C-X-C motif) receptor 2 (CXCR2), was compared in unaffected lung tissue and biopsies from dogs affected with CIPF by quantitative PCR and localisation of CCL2 and CXCL8 proteins were determined by immunohistochemistry.

Significantly greater CCL2 and CXCL8 concentrations were found in the BALF from WHWTs affected with CIPF, compared with healthy dogs. Significantly greater serum concentrations of CCL2, but not CXCL8, were found in CIPF-affected dogs compared with healthy WHWTs. No differences in relative gene expression for CCL2, CXCL8, CCR2 or CXCR2 were observed when comparing lung biopsies from control dogs and those affected with CIPF. In affected lung tissues, immunolabelling for CCL2 and CXCL8 was observed in bronchial airway epithelial cells in dogs affected with CIPF. The study findings suggest that both CCL2 and CXCL8 are involved in the pathogenesis of CIPF. Further studies are required to determine whether these chemokines might have a clinical use as biomarkers of fibrosis or as targets for therapeutic intervention.

Introduction

Canine idiopathic pulmonary fibrosis (CIPF) is a progressive disease of the lung parenchyma, which mainly affects older dogs of the West Highland white terrier (WHWT) breed (Heikkila-Laurila and Rajamaki, 2014). The aetiology and pathogenesis of CIPF remain to be established, but a genetic basis is strongly suspected due to the breed predisposition (Heikkila-Laurila and Rajamaki, 2014).

CIPF shares several clinical features with human idiopathic pulmonary fibrosis (HIPF) (Corcoran et al., 1999; Lobetti et al., 2001; Heikkila et al., 2011), although there are some histopathological differences between the two syndromes (Syrja et al., 2013). As in HIPF, progression of the disease in dogs can vary considerably, with survival times from onset of clinical signs ranging from around 2 months to 4 years, with a median of 2.7 years (Raghu et al., 2011; Lilja-Maula et al., 2014).

There is no effective therapy available for CIPF at the present time (Heikkila-Laurila and Rajamaki, 2014) and even in HIPF, only two drugs, pirfenidone and nintedanib, have demonstrated a degree of efficacy in slowing progression of the disease (Covvey and Mancl, 2014). In veterinary medicine, the response to specific anti-fibrotic treatment has not yet been determined and few assays are currently available for monitoring progression and, consequently, treatment efficacy for CIPF. Arterial blood gas analysis and the 6-min walking test can be used for evaluation of lung function in dogs affected with CIPF, but these tests may lack sensitivity (Lilja-Maula et al., 2014). There is, therefore, a need for specific biomarkers that could help in determining the severity of lung dysfunction and monitoring disease progression.

Chemokine (C-C motif) ligand 2 (CCL2), also known as monocyte chemoattractant protein (MCP)-1, has been studied extensively in HIPF. Elevated CCL2 concentrations can be detected in bronchoalveolar lavage fluid (BALF) (Capelli et al., 2005; Baran et al.,

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Table 1
Details of West Highland white terriers (WHWTs) affected with CIPF included in the study.

Case number	Signalment		Diagnostic procedures		Experimental procedures			
	Age at presentation	Gender	Lung HRCT	Lung histology	Serum ELISA	BALF ELISA	Lung qPCR	Lung IHC
1	9 years 10 months	M	+	–	–	+	–	–
2	14 years 6 months	M	+	+	–	+	–	–
3	10 years 5 months	F	+	–	+	+	–	–
4	10 years 6 months	F	+	–	+	+	–	–
5	11 years 7 months	M	+	+	+	+	–	–
6	12 years 4 months	M	+	–	+	+	–	–
7	13 years 11 months	M	+	+	+	+	–	–
8	11 years 5 months	M	+	–	+	+	–	–
9	10 years 11 months	M	+	–	+	+	–	–
10	5 years 2 months	F	+	–	+	+	–	–
11	13 years 11 months	M	+	+	+	–	–	–
12	10 years 5 months	M	+	–	+	–	–	–
13	11 years	M	–	+	+	–	–	+
14	11 years 8 months	F	+	+	+	+	+	–
15	11 years 7 months	M	+	+	+	+	+	+
16	8 years 2 months	M	–	+	+	–	+	–
17	14 years 2 months	M	–	+	–	–	+	+
18	11 years 5 months	F	–	+	–	–	+	+
19	12 years 5 months	F	–	+	–	–	+	–
20	12 years 1 months	M	–	+	–	–	+	–
21	11 years 9 months	M	+	+	–	–	+	–
22	15 years 1 months	F	–	+	–	–	+	–
23	8 years 11 months	F	+	+	–	–	+	–
24	10 years 9 months	F	+	+	–	–	+	–
25	8 years 11 months	M	–	+	–	–	+	–
26	11 years 4 months	F	+	+	–	–	+	–
27	11 years 8 months	M	+	+	–	–	+	–
28	9 years 7 months	F	+	+	–	–	+	–
29	12 years 6 months	F	–	+	–	–	+	–
30	11 years 4 months	M	–	+	–	–	+	–
31	15 years	F	+	+	–	–	+	–

HRCT, high resolution computed tomography; ELISA, enzyme-linked immunosorbent assay; qPCR, quantitative polymerase chain reaction; IHC, immunohistochemistry.

2007) and blood (Suga et al., 1999; Fujiwara et al., 2012) of patients affected with HIPF and correlate with clinical parameters of lung function (Capelli et al., 2005; Emad and Emad, 2007) and outcome (Shinoda et al., 2009). CCL2 acts via (C-C motif) receptor 2 (CCR2), which is expressed on numerous cell types (Bonecchi et al., 2009). In HIPF, CCL2 acts on pulmonary fibroblasts, leading to synthesis of abundant extracellular matrix, via expression of transforming growth factor (TGF)-β1, a potent pro-fibrotic mediator (Gharaee-Kermani et al., 1996). CCL2 also contributes to lung pathology through recruitment of circulating fibrocytes (Moore et al., 2005), which produce type I collagen (Moore et al., 2005) and which differentiate into fibroblasts and myofibrobasts during the fibroproliferative process observed in HIPF (Phillips et al., 2004). A recent study in a murine model showed that pirfenidone significantly improved lung fibrosis, through attenuation of CCL2 production and reduced fibrocyte recruitment (Inomata et al., 2014), suggesting that CCL2 might be useful as a biomarker of fibrosis as well as a target for therapeutic intervention.

Chemokine (C-X-C motif) ligand 8 (CXCL8), also known as interleukin (IL)-8, is also found in increased concentration in the BALF (Antoniou et al., 2006) and serum (Ziegenhagen et al., 1998b) of patients with HIPF and correlates with lung function (Martina et al., 2009; Vasakova et al., 2009), disease progression (Ziegenhagen et al., 1998a; Totani et al., 2002) and survival (Richards et al., 2012). The role of CXCL8 in the pathogenesis of HIPF is not well understood, but several authors have suggested that CXCL8 might act as a pro-fibrotic factor, via promotion of angiogenesis through chemokine (C-X-C motif) receptor 2 (CXCR2) (Antoniou et al., 2006; Martina et al., 2009; Cui et al., 2010). Furthermore, a single nucleotide polymorphism (rs4073T>A) has been identified in the promoter region of the CXCL8 gene, which has been shown to be significantly associated with increased risk of developing HIPF (Ahn et al., 2011).

Analysis of gene expression in lung samples from CIPF-affected dogs has revealed increased expression of several genes, including those encoding CCL2 and CXCL8 (Krafft et al., 2013). The same study showed that serum CCL2 concentrations were elevated in dogs affected with CIPF, compared with healthy controls of the same breed (Krafft et al., 2013). The aim of the present study was to further evaluate the role of chemokines CCL2 and CXCL8 in the pathogenesis of CIPF, with a view to determining whether these chemokines might have potential as biomarkers of pulmonary fibrosis.

Materials and methods

Study population and samples

A total of 31 WHWTs affected with CIPF and 41 unaffected control dogs, including 20 WHWTs and 21 dogs of other breeds, were included in the study (Tables 1 and 2). Dogs were recruited at the University of Liège, the University of Helsinki, and by other partners engaged in the European CIPF project.[1] The study was approved by the Committee of Experimental Animals of Western Finland (approval numbers: ESLH-2008-05403, date of approval: 27 June 2008; ESAVI/7383/04.10.07/2013, date of approval: 13 November 2013) and by the equivalent committee of the University of Liège, Belgium (approval number: 1435, date of approval: 14 March 2013). All samples were obtained with informed owner consent.

The diagnostic approach for CIPF has been described elsewhere (Heikkila et al., 2011; Syrja et al., 2013) and the diagnosis was confirmed either by thoracic high-resolution computed tomography (HRCT; $n = 8$), lung histopathology ($n = 10$) or by both methods ($n = 13$). The health status of unaffected WHWTs used as controls for serum and BALF measurements (Table 2A, $n = 18$) was assessed by taking a complete history, performing a physical examination and by performing haematology and serum biochemistry. In nine of these dogs, thoracic HRCT was also performed at the time of sampling, which did not reveal any abnormalities. Lung tissues used as controls for quantitative PCR and immunohistochemistry procedures (Table 2B, $n = 23$) were

[1] See: http://www.caninepulmonaryfibrosis.ulg.ac.be/vet-partners/ (accessed 01.06.15).