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Short communication

Serum levels of innate immunity cytokines are elevated in dogs with metaphyseal osteopathy (hypertrophic osteodytrophy) during active disease and remission

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ABSTRACT

Metaphyseal osteopathy (MO) (hypertrophic osteodystrophy) is a developmental disorder of unexplained etiology affecting dogs during rapid growth. Affected dogs experience relapsing episodes of lytic/sclerotic metaphyseal lesions and systemic inflammation. MO is rare in the general dog population; however, some breeds (Weimaraner, Great Dane and Irish Setter) have a much higher incidence, supporting a hereditary etiology. Autoinflammatory childhood disorders of parallel presentation such as chronic recurrent multifocal osteomyelitis (CRMO), and deficiency of interleukin-1 receptor antagonist (DIRA), involve impaired innate immunity pathways and aberrant cytokine production. Given the similarities between these diseases, we hypothesize that MO is an autoinflammatory disease mediated by cytokines involved in innate immunity. To characterize immune dysregulation in MO dogs we measured serum levels of inflammatory markers in 26 MO and 102 control dogs. MO dogs had significantly higher levels (pg/ml) of serum Interleukin-1beta (IL-1β), IL-18, IL-6, Granulocyte-macrophage colony stimulating factor (GM-CSF), C-X-C motif chemokine 10 (CXCL10), tumor necrosis factor (TNF), and IL-10. Notably, recovered MO dogs were not different from dogs during active MO disease, providing a suggestive mechanism for disease predisposition. This is the first documentation of elevated immune markers in MO dogs, uncovering an immune profile similar to comparable autoinflammatory disorders in children.

1. Introduction

Metaphyseal osteopathy (MO) is an inflammatory bone disease characterized by metaphyseal necrosis, hemorrhage and inflammation (Joiner and Montgomery, 2011). The disease affects young dogs during the peak of their growth and diminishes after closure of the growth plates (Grondalen, 1976; Munjar et al., 1998). Clinically, dogs present with non-specific multisystemic signs of inflammation including fever, ocular and nasal discharge, skin pustules, diarrhea, hematochezia, vomiting, vulvovaginitis, and inflammation involving the airways (Abeles et al., 1999; Safra et al., 2012). The hematological and serum biochemical findings of MO are non-specific but often indicate inflammation (Safra et al., 2012). Radiographs reveal lytic sclerotic lesions in the metaphyses of multiple long bones, which on metaphyseal biopsies demonstrate neutrophilic inflammation, hemorrhage and necrosis (Franklin et al., 2008; Miller, 2001).

Treatment of MO consists of non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids, to decrease immune activation and inflammation. Individuals that respond poorly to NSAIDs often respond favorably to immunosuppressive doses of corticosteroids (Abeles et al., 1999; Safra et al., 2012). Finally, relapses during the growth period are common, especially in dogs with multi-organ involvement (Safra et al., 2012). The overall picture supports an idiopathic chronic inflammatory disease similar to immune-mediated steroid-responsive meningitis arteritis (Biedermann et al., 2016), granulomatous meningoencephalitis (Park et al., 2013), immune-mediated polyarthropathy (Foster et al., 2014), and inflammatory bowel disease (Maeda et al., 2016) in dogs.

The underlying etiology of MO remains unknown. Several mechanisms have been proposed, including nutritional, infectious and vaccine induced causes, although no substantial evidence support-

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ing these theories has been identified (Meier et al., 1957; Munjar et al., 1998; Safra et al., 2012). A hereditary component for MO is evidenced by predisposition of specific breeds such as Great Danes, Boxers, German Shepherd Dogs, Irish Setters, and Weimaraners (Harrus et al., 2002; Miller, 2001; Munjar et al., 1998). MO in littermates and closely related dogs has been reported for Weimaraners (Abeles et al., 1999; Safra et al., 2012), Australian Kelpies (Greenwell et al., 2014) and Irish Setters (Brown, 2007).

In children, chronic recurrent multifocal osteomyelitis (CRMO) is a complex autoinflammatory disease that closely resembles MO in its clinical presentation (Ferguson and Laxer, 2015; Sharma and Ferguson, 2013). CRMO patients typically present with recurrent episodes of inflammation involving the bone, skin, and occasionally the intestines. The inflammation is sterile and autoantibodies are absent, suggesting a dysregulation of innate immunity pathways (Chitu et al., 2009). A form of CRMO with a known molecular basis is deficiency of interleukin-1 receptor antagonist (DIRA). Due to oversignaling of IL-1, DIRA patients experience systemic inflammation, sterile lytic bone lesions and pustulosis (Moghaddas and Masters, 2015). A naturally occurring model to CRMO is the chronic multifocal osteomyelitis (cmo) mouse. Due to a homozygous mutation in the proline-serine-threonine phosphatase interacting protein 2 (Pstpip2), cmo mice develop sterile multifocal osteomyelitis and extramedullary hematopoiesis with occasional inflammation of the skin (Chitu et al., 2009). The human analog of Pstpip2 is a phosphatidate phosphatase gene named Lipin 2, defects of which also cause CRMO. CRMO is marked by a pro-inflammatory cytokine profile with elevated serum levels of IL-1β, IL-6 and TNF (Stern and Ferguson, 2013).

Given these findings, we hypothesize that MO is an autoinflammatory disorder characterized by increased levels of cytokines involved in innate immunity, similar to CRMO patients and cmo mice. To test this hypothesis, we performed a prospective descriptive study of serum levels of inflammatory markers in MO dogs and controls.

2. Materials and methods

2.1. Dogs

26 MO cases (9 Irish Setters and 17 Weimaraners, Table S1 in the online version, at http://dx.doi.org/10.1016/j.vetimm.2016.08.003) were included on the basis of signalment; clinical signs of pyrexia, lethargy, and ostealgia; and radiographic evidence of MO as evaluated by a board-certified veterinary radiologist (EGJ). Complete medical records and copies of the diagnostic radiographs were required for inclusion. Information obtained from the medical records consisted of signalment, clinical signs, vaccination history, treatment, response to treatment, and relapse episodes. Eight of the dogs were in remission (4 puppies and 4 adults), defined by absence of clinical signs and observed levels of activity appropriate to age. Individuals were assessed to be in remission if they had an unremarkable physical examination documented in the medical record, and had no gait abnormalities as assessed through review of video material and owner interviews. Participation of dog owners was solicited with announcements posted on the Weimaraner Club of America and Irish Setter Club of America websites, by direct communication with dog breeders, owners, attending veterinarians of MO cases, and via the Veterinary Information Network. 102 control dogs free of MO (according to medical records) were included, consisting of 34 unaffected Irish Setter dogs, 52 laboratory mixed-breed dogs (Marshall Farms USA, Inc.), and 16 mixed-breed shelter dogs. Dogs of both sexes and of various ages were included (Table S1 in the online version, at http://dx.doi.org/10.1016/j.vetimm.2016.08.003).

2.2. Serum markers

Whole blood samples were collected in serum collection tubes (red or tiger top). Clotted blood was separated by centrifugation and the serum was aspirated and stored in 1.5 ml eppendorf tubes at -20◦ C until the time of analysis. Each sample was analyzed once. Serum levels of 13 cytokines (GM-CSF, IFN-γ, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, CXCL10/IP-10, KC-like, CCL2/MCP-1and TNF), were determined using MILLIPLEX MAP Canine Cytokine/Chemokine Magnetic Bead Panel by EMD Millipore (Billerica Massachusetts, USA). The multiplex includes cytokines of interest that were previously implicated in autoinflammatory disorders, and was validated for canine serum. The samples were incubated according to protocol and reading was performed using a Bio-Plex 200 Multiplex System at the UC Davis Human Immune Monitoring Core. Serum levels of canine IL-1 β were evaluated because of its involvement in CRMO, and were determined by ELISA (RayBiotech, Inc. Norcross, Georgia, USA), following the kit protocol. Cytokine functions are summarized in Table S2 in the online version, at http://dx.doi.org/10.1016/j.vetimm.2016.08.003.

2.3. Statistical analysis

Shapiro-Wilk test for normal distribution of serum levels of immune markers (pg/ml) was performed for each of the markers. Since none of the marker levels (including log-transformed levels) were normally distributed, the Wilcoxon rank-sum (Mann–Whitney) test was used to compare marker levels between MO affected and unaffected dogs. Analyses of affected *versus* unaffected dogs were conducted for all dogs combined, as well as stratified by sex, breed, and age group (adult, puppy). Logistic regression was used to estimate odds ratios (OR), defined as the relative odds of being affected *versus* non-affected, and displaying 95% confidence intervals. Marker levels were log-transformed for the regression analysis. Models were adjusted for age (puppy/adult), sex (female/male), and remission status (yes/no). Data were statistically analysed using Stata (StataCorp. 2013. Stata Statistical Software: Release 13.1. College Station, TX: StataCorp LP.)

3. Results and discussion

All confirmed MO dogs (11 females and 15 males) had their first episode during accelerated growth of long bones, between the ages of 7–18 weeks. Clinical signs consisted of ostealgia (n = 26), pyrexia (n = 24), depression (n = 24), anorexia (n = 22), diarrhea (n = 16), vulvo-vaginitis (n = 7), pustules (n = 5), hematochezia (n = 4), vomiting (n = 4), abnormal lung sounds (n = 4), sore jaw (n = 4), ocular (n = 4) and nasal (n = 3) discharges. Twenty dogs were vaccinated within 30 days prior to a MO episode, and 10 dogs experienced 2–8 relapses.

Six dogs responded to initial treatment with NSAIDs (carprofen (Rimadyl; Zoetis, USA or Vetprofen; Vetoquinol USA, 4.4 mg/kg (2 mg/lb), q 24 h for 7 days (n = 4); firocoxib) (Previcox; Merial USA, 5 mg/kg (2.27 mg/lb), q 24 h for 7 days (n = 1); meloxicam (Metacam; Boehringer Ingelheim Vetmedica, USA) 0.1 mg/kg (0.045 mg/lb), q 24 h for 7 days (n = 1)), and 10 dogs responded to initial treatment with prednisone (makers not specified), 0.75–1.5 mg/kg (0.34–0.68 mg/lb), q 12 h for 7 days, followed by a tapering dose or as needed. Ten dogs were NSAID-resistant, and all of them responded positively to a change of treatment to prednisone.

The results of the statistical analysis comparing serum levels of cytokines between MO dogs and controls are summarized in Table 1 and Fig. S1 in the online version, at http://dx.doi.org/10.1016/j.vetimm.2016.08.003. Compared with control dogs,