

Acute Phase Response with Special Reference to C-Reactive Protein in Dogs with Generalized Demodicosis

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ABSTRACT

Background: Canine demodicosis is an inflammatory parasitic skin disease associated with the presence of excessive numbers of *Demodex* mites. The pathogenesis involving demodicosis is not completely recognized. It is suggested that demodicosis occurs because of the inability of the host to regulate mite intensity, rather than from an elevation of the mite virulence. The acute phase response is a part of the innate defence system of the host against trauma, inflammation, and infection. Some of the cytokines involved in pathogenic phenomena in canine demodicosis are known as inducers of the acute phase response. Among the large group of acute phase reactants, C-reactive protein (CRP) may have special attention in canine demodicosis, because of its relationship with cytokines involved in the development of skin inflammation. In this study we sought to elucidate the relationship between generalized demodicosis and the serum concentration CRP in dogs.

Materials, Methods & Results: Twenty two dogs (n = 14 demodicosis, n = 8 healthy), enrolled in the study were recruited from the dogs referred to 5 different veterinary practices participated. Dogs of various breed, both sexes, entire/neutered were included in the study on the basis of being *Demodex canis* mite positive on microscopic examination of skin scrapings. Cytological examination of the skin scraping showed no yeast nor bacteria. The animals exhibited at least one of the following clinical signs of generalized demodicosis: presence of = 5 spot lesions, involvement of = 25% of the dog's face or of its body surface, pododemodicosis involving two or more feet. None of the dogs completing the study had been treated with ectoparasiticides or steroidal anti-inflammatory drugs in the last 30 days before the onset of the study. Plasma CRP concentration was determined using commercial ELISA test kit and an ELISA reader according to the manufacturer instructions. Statistical analysis were performed by Mann-Whitney U test. P values of less than 0.05 were regarded as significant. Serum CRP concentration was significantly higher in dogs with generalized demodicosis compared to those of control dogs

Discussion: The present study presents for the first time a survey of the acute phase protein expression regarding CRP in serum of dogs affected by demodicosis. Results indicated that the concentration of investigated, including serum CRP, significantly increased in infected dogs. From a clinical perspective, acute phase protein measurement is a useful clinical tool in veterinary medicine but further studies are required to assure their value in particular diseases, because the acute phase response shows variation in different species and pathological processes. The mean plasma levels of the C-reactive protein measured in the peripheral blood of the 14 dogs included in the present study with generalised demodicosis were significantly elevated compared to those of the healthy controls. The results indicated that in generalised demodicosis acute phase response can be mobilized. According to results of this study, we thought that Demodectic mite may have the potential to stimulate an inflammatory response in dogs with generalized demodicosis. This information provides a remarkable potential for use in clinical practice for dogs, hence these biological markers may be very useful for the early diagnosis of inflammation due to the demodectic mange. Although the numbers of dogs investigated in this study was small, CRP concentration could be beneficial in monitoring the response to treatment in dogs with generalized demodicosis. Further studies are warranted for evaluating the clinical usage of this parameter for monitoring the efficacy of therapy in dogs with demodicosis.

Keywords: Acute phase protein, C-reactive protein, generalised demodicosis, dog.

INTRODUCTION

Canine demodicosis is an inflammatory parasitic skin disease associated with the presence of excessive numbers of *Demodex* mites [6,11]. *Demodex* mites reside within hair follicles and, less commonly, sebaceous glands of the normal canine skin. Factors responsible for excessive proliferation of this normal skin commensal mite in dogs are not very well known, however genetic or acquired disorders influencing immunity have been suggested to play a major role [11].

Demodicosis is classified as either localized or generalized regarding the extent of the disease. Localized demodicosis is a benign disease that mostly resolves spontaneously and is not normally associated with concurrent bacterial pyoderma. In contrast, generalized demodicosis is considered one of the most severe canine skin diseases and mostly involves secondary bacterial skin infections, which require administration of systemic antibiotics concomitantly with acaricidal treatment [8,9].

The pathogenesis involving demodicosis is not completely recognized. It is suggested that demodicosis occurs because of the inability of the host to regulate mite intensity, rather than from an elevation of the mite virulence. There is evidence suggesting that excessive proliferation of the *Demodex* mites in the affected dogs may be related with an underlying genetic or immunological disorder and they may be prone to develop demodicosis on a hereditary basis [12,13].

The acute phase response is a part of the innate defence system of the host against trauma, inflammation, and infection [5,10]. Some of the cytokines involved in pathogenic phenomena in canine demodicosis are known as inducers of the acute phase response. Among the large group of acute phase reactants, C-reactive protein (CRP) may have special attention in canine demodicosis, because of its relationship with cytokines involved in the development of skin inflammation [15]. CRP binds the changed biological material in peripheral blood, and may be functioning within blocking, detoxication and facilitation of elimination [14]. Elevated CRP concentration in the peripheral blood and other body fluids is due to the interaction between proin-

flammatory cytokines, their receptors and inhibitory factors [5]. Therefore, the changes in CRP concentrations in blood observed in various stages of disease activity are indirect indications of the participation of these cytokines in the pathogenic process [5].

Limited data on acute phase response in canine demodicosis infection exist. In this study we sought to elucidate the relationship between generalized demodicosis and the serum concentration CRP in dogs. Therefore the present study was carried to gain insight into the acute phase reaction during *Demodex* infection in dogs. CRP was selected because it is one of the most common acute phase protein in dogs.

MATERIALS AND METHODS

Identification of dogs and clinical features

Twenty two dogs (n = 14 demodicosis, n = 8 healthy), enrolled in the study were recruited from the dogs referred to 5 different veterinary practices participated. Dogs of various breed, both sexes, entire/neutered were included in the study on the basis of being *Demodex canis* mite positive on microscopic examination of skin scrapings. Cytological examination of the skin scraping showed no yeast nor bacteria. The animals exhibited at least one of the following clinical signs of generalized demodicosis: presence of = 5 spot lesions, involvement of = 25% of the dog's face or of its body surface, pododemodicosis involving two or more feet. Pregnant or lactating females were excluded. The dogs were kept under their routine housing conditions and were given their normal feed. None of the dogs completing the study had been treated with ectoparasiticides or steroidal anti-inflammatory drugs in the last 30 days before the onset of the study.

Determination of serum CRP

Blood samples were taken from the cephalic vein into heparinized tubes. Plasma was separated by centrifugation at 1700 g for 10 min and kept at -20°C until analysis. Plasma CRP concentration was determined using commercial ELISA test kit¹ and an ELISA reader² according to the manufacturer instructions.

Statistical analysis

Statistical analysis were performed by Mann-Whitney U test. *P* values of less than 0.05 were regarded as significant.

RESULTS

Serum concentration of acute phase protein expressed as serum CRP concentration was determined on 8 healthy dogs and 14 dogs with generalized demodicosis. Results were presented in Table 1. Serum CRP concentration was significantly higher in dogs with generalized demodicosis compared to those of control dogs (Table 1).

DISCUSSION

The present study presents for the first time a survey of the acute phase protein expression regarding CRP in serum of dogs affected by demodicosis. Results indicated that the concentration of investigated, including serum CRP, significantly increased in infected dogs.

From a clinical perspective, acute phase protein measurement is a useful clinical tool in veterinary medicine but further studies are required to assure their value in particular diseases, because the acute phase response shows variation in different species and pathological processes. CRP is reported

Table 1. Plasma concentration of C-reactiv protein in dogs with healthy and generalised demodicosis.

		n	min	max	Mean	SEM	<i>P</i>
CRP (µg/mL)	Demodicosis	14	7,87	69,54	38,33	5,50	< 0,001
	Healthy	8	0,87	14,00	7,60	2,01	

to be the major acute phase protein in dogs [14]. CRP concentrations in healthy dogs are reported as 0.8-14.0 µg/mL, without any sex predilection [7]. In dogs, plasma concentrations of CRP, serum amyloid-A and haptoglobin are increased in many infectious and inflammatory conditions, such as surgical trauma, polyarthritis, leptospirosis, bacterial and hemorrhagic enteritis, parvoviral infection, ehrlichiosis, and leishmaniasis [4]. The mean plasma levels of the C-reactive protein measured in the peripheral blood of the 14 dogs included in the present study with generalised demodicosis were significantly elevated compared to those of the healthy controls (Table 1). The results indicated that in generalised demodicosis acute phase response can be mobilized.

Canine demodicosis is classified as either localized or generalized depending on the extent of skin lesions, and their immunological responses differ. Generalized demodicosis is considered one of the most severe canine skin diseases and mostly involves secondary bacterial skin infections. The decrease in the mitogen-stimulated response of peripheral blood mononuclear cells is more severe in generalised demodicosis than in localized demodicosis [2].

Cytokines play an important role in development of immune response and its regulation [3], and thus cytokine profiles contribute to the effect of immunity level in the diseases. Some of the cytokines involved in pathogenesis in canine demodicosis are known as inducers of the acute phase response [15]. The vesicle-like follicular pustules are lesions of clinically active demodicosis and are characterized by infiltration of the follicular epithelium by CD3+ and CD8+ T lymphocytes. These lymphocytes are cytotoxic T cells, which may cause an injury to the follicular epithelium in case of demodicosis [1]. We consider it important that our group of subject evidenced elevated concentration of CRP during the course of active disease. Change in CRP activity indirectly confirm participation of proinflammatory cytokines in the generalised demodicosis process. The follicular epithelium contain a considerable number of inflammatory cells, including T lymphocytes, macrophages, neutrophils, and eosinophils. Inflammatory cytokines (eg. tumor necrosis factor [TNF]-α, interleukin [IL]-1, IL-6 are released from these cells in tissue damage. In our study, the significant change in plasma CRP concentration might

be related to the release of inflammatory cytokines from follicular epithelium as a result of the inflammatory response in the affected lesional sites.

According to results of this study, we thought that Demodectic mite may have the potential to stimulate an inflammatory response in dogs with generalized demodicosis. This information provides a remarkable potential for use in clinical practice for dogs, hence these biological markers may be very useful for the early diagnosis of inflammation due to the demodectic mange. Although the numbers of dogs investigated in this study were small, CRP concentration could be beneficial in monitoring the

response to treatment in dogs with generalized demodicosis. Further studies are warranted for evaluating the clinical usage of this parameter for monitoring the efficacy of therapy in dogs with demodicosis.

SOURCES AND MANUFACTURERS

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