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Evaluation of the expression of P-selectin, ICAM-1, and TNF-alpha in bacteria-free lesional skin of atopic dogs with low-to-mild inflammation

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Abstract

Canine atopic dermatitis (AD) is a pruritic skin condition that shares many clinical and pathophysiological features with its human counterpart. A major therapeutic challenge of AD is the control of the skin inflammatory process. A detailed knowledge of the pro-inflammatory molecules involved in cell recruitment in AD would allow for a better control of the disease. We thus have studied the protein expression of P-selectin, ICAM-1 and TNF-α in the lesional and non-lesional skin of atopic dogs that had been treated for bacterial infections. Despite a low-to-mild inflammatory process, P-selectin protein was clearly upregulated in the lesional skin areas when compared with non-lesional skin (four-fold average increase). This P-selectin upregulation was accompanied by signs of functional changes such as increased cell margination, and membrane-associated protein expression. Although the expression of ICAM-1 and TNF-α was not enhanced in the lesional *versus* the non-lesional skin, there was a trend towards a correlated upregulation of both molecules. Further studies will help elucidate the significance of the substantial overexpression of P-selectin in canine AD, in particular in a scenario where bacterial antigens are not contributing as pro-inflammatory stimuli.

Keywords: Adhesion molecules; Atopic dermatitis; Cytokines; Inflammation

1. Introduction

Atopic dermatitis (AD) is a pruritic skin disease commonly recognised in humans and dogs. Human and canine AD share many features (Willemse, 1988; Griffin and DeBoer, 2001), and these similarities have advocated the use of dog systems as models for furthering into human AD (reviewed in de Mora et al., 2006). Although the precise immunological mechanisms of such cutaneous disorder are unclear, in both species a late phase reaction (LPR) with an underlying

Abbreviations: Ab, antibody; ABC, avidin/biotin complex; AD, atopic dermatitis; BOG, bacterial overgrowth; DAB, 3,3-diaminobenzidine; HPF, high power field; ICAM-1, intercellular adhesion molecule-1; OCT, Tissue-Tek® O.C.T. Compound; TNF-α, tumor necrosis factor-α

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inflammatory process develops. A key aim in AD research is to uncover the cellular and molecular elements that contribute to such persistent inflammation. Many groups have characterized the dermal inflammatory infiltrate of human (Leung, 1999), and canine (Olivry et al., 1997; Sinke et al.,1997) AD, but controversy arises as to what cells are primarily involved. Several pro-inflammatory molecules are potential candidates to coordinate this cell recruitment. Histamine appears to be increased in the skin of dogs (Nimmo Wilkie et al., 1990; de Mora et al., 1996), and humans (Ruzicka and Gluck, 1983; Sampson et al., 1989) with AD. This mediator is suggested to upregulate *in vitro* the expression of P-selectin (Jones et al., 1993). This might explain why P-selectin is one of the adhesion molecules associated to AD inflamed sites in human skin (Wakita et al., 1994; Barker, 1995; Jung et al., 1996), and also in experimental settings of *in vivo* murine IgE-mediated dermatitis (de Mora et al., 1998). A report from Chenier and Dore (1998) described increased protein levels of P-selectin in different inflammatory canine skin diseases, and found a positive correlation with the amount of infiltrating cells. However, no data on its expression pattern in canine AD were provided. In addition, high levels of free and membrane-associated intercellular adhesion molecule-1 (ICAM-1) have been found in human patients with atopic asthma and dermatitis (Barker, 1995; Jung et al., 1996). This molecule participates in the adhesion phase of the migration process, i.e. subsequent to the selectins-induced rolling. Olivry et al. (1997) suggested neo-expression of this adhesion molecule in the epidermal basal layer, and upregulation in endothelial cells in lesional skin samples of dogs with AD. A major upregulator of ICAM-1 is the pro-inflammatory cytokine tumor necrosis factor (TNF)-α, which has been largely associated to clinical and experimental allergy (de Vries et al., 1998; Junghans et al., 1998). At the mRNA level, canine TNF-α is overexpressed in the lesional skin of dogs (Olivry et al., 1999; Maeda et al., 2002; Nuttall et al., 2002), and humans (Grewe et al., 1998; Leung, 1999) with AD, but a complementary analysis of this cytokine's protein expression in lesional and non-lesional skin of dogs with AD is also of interest.

In order to assess the expression of the pro-inflammatory proteins P-selectin, ICAM-1 and TNF-α in the skin of atopic dogs, lesional and non-lesional biopsies were collected from selected atopic dogs that had been managed for dermal infections.

2. Materials and methods

2.1. Atopic dogs inclusion criteria

Six mixed-breed atopic dogs were selected from patients of the Veterinary Hospital at the *Universidad Autónoma de Barcelona*. Diagnosis was based on the presence of at least three major and three minor features (see legend under Table 1 for a list of the diagnostic criteria) as proposed by Willemse (1986). The presence of elevated allergen-specific IgE levels in serum (Allercept, Univet, Spain) was always among the minor features of the selected animals. All dogs were fed an 8-week hypoallergenic Purina restriction diet to rule out adverse food reactions. They underwent also flea control and were treated, if needed, for yeast and/or fungi infections. A cytological examination to detect *Malassezia*, bacterial overgrowth (BOG), and/or superficial bacterial infection (through the scraping technique), was carried out in each dog. BOG was considered positive if under the scotch test, the number of cocci per 100× microscope field (immersion objective) exceeded 10. As for the dermatological examination, most of the animals (five out of six) had superficial staphylococcal pyoderma at the first visit. They were treated with Cephalexin (Rilexine 300, Virbac, 1 tablet/20 kg b.i.d.)

Table 1
Summary of the most relevant data about the clinical and the histopathological diagnostic of the six selected atopic dogs

Dog ref.	Diagnostic tests			Skin biopsy analysis	
	Allercept[a]	Major features[b]	Minor features[c]	Level of inflammation	Hyperkeratosis and follicular keratosis
1	15	a, b, c, e	f, h, j	Low	Hyperplasic perivascular dermatitis
2	2	a, b, c, e	f, h, j	Low	Hyperplasic perivascular dermatitis
3	3	a, b, c, e	f, g, j	Medium	Hyperplasic perivascular dermatitis
4	24	a, b, c,	f, h, j	Medium	Hyperplasic perivascular dermatitis
5	4	a, d	h, j	Medium	Hyperplasic perivascular dermatitis
6	4	a, c	h, i, j	Medium	Hyperplasic perivascular dermatitis

[a] Number of allergens towards which specific IgE was detected.
[b] a: pruritus, b: facial and/or digital involvement, c: chronic dermatitis, d: history of atopy, e: breed predilection.
[c] f: signs before 3 years g: facial erythema and chelitis, h: superficial pyoderma, i: hyperhydrosis and j: IgE (allercept+).