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The ACVD task force on canine atopic dermatitis (XII): the relationship of cutaneous infections to the pathogenesis and clinical course of canine atopic dermatitis

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Abstract

Dogs and human beings with atopic dermatitis (AD) frequently exhibit concurrent skin infections with *Staphylococcus* sp. bacteria or *Malassezia* yeast, and treatment of such infections is an important facet of managing these patients. Staphylococci appear to colonize atopic skin readily, and bacterial products on the skin could augment cutaneous inflammation via immediate hypersensitivity responses to the bacteria, by superantigen-mediated lymphocyte activation, or other non-specific mechanisms. Similarly, skin colonization by *Malassezia* yeast could contribute to clinical signs of AD; yeast components could induce inflammation via non-specific mechanisms, such as alteration in mediator release, or via antigen-specific hypersensitivity reactions. Clinical and experimental evidence exists that secondary microbial infections can both initiate and perpetuate episodes of AD in dogs and humans, and could even participate in promotion of pro-allergic immunologic responses. Mechanistic details of these complex interactions are under extensive investigation in human beings; only a few observations have been extended to include dog with AD.
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1. Introduction

Cutaneous infections, in particular those of a recurrent nature, commonly are observed in dogs and humans with atopic dermatitis (AD). The relationship of these infections to the

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pathogenesis and clinical signs of AD is complex, and in most cases not completely clear. Skin infections could, in some cases, be a consequence of changes in the skin brought about by the AD itself (e.g. self-induced excoriations). Conversely, there is abundant evidence to suggest that these infections are an important component of the pathogenesis of AD through their effects on the immune system and/or by perpetuating the cutaneous inflammatory response. Regardless of their cause-and-effect relationship, it is clear that managing such infections is an important and integral part of overall treatment of the patient with AD.

Human patients with AD suffer from an increased susceptibility to infections with a variety of microorganisms, including staphylococci, *Malassezia* or *Candida* yeast, dermatophytes, and viruses (Jones et al., 1973; Rystedt et al., 1986; Ring et al., 1992). Of these organisms, only staphylococci and yeast have been associated with canine AD. This review will therefore focus on current knowledge of the relationship between staphylococcal or yeast infections and AD in dogs and human beings.

2. Staphylococcal infections and atopic dermatitis in humans and dogs

Recurrent staphylococcal infections have a strong association with human AD and are an important clinical aspect of this disease. The skin of greater than 90% of human patients with AD is heavily colonized with *Staphylococcus aureus*, and such colonization is usually not reversed even with prolonged antimicrobial treatment. Superficial infections (folliculitis and impetigo) are typical, with an apparent resistance to deeper extension (Hanifin, 1992). The skin lesions of human patients with AD respond to treatment better when antibiotics are added to the regimen, even when overt signs of staphylococcal infection are not evident (Leyden and Kligman, 1977).

The clinical signs of staphylococcal skin infection in dogs are varied; for a complete review, the reader is referred to Scott et al. (2001). Certainly, veterinary dermatologists agree that in dogs, staphylococcal infections themselves result in inflammation and mild to severe pruritus, even without the presence of AD; when AD is present concurrently, the additional pruritic stimulus can induce even more patient discomfort. Antibiotic treatment of canine AD patients with concurrent infections produces anywhere from a partial to a dramatic reduction in clinical signs. Some clinicians believe that canine AD can be manifested solely as recurrent staphylococcal pyoderma that is completely antibiotic-responsive, i.e. where the dog remains non-pruritic and without skin lesions as long as the infection is controlled (Scott et al., 2001). In these dogs, it is believed that the frequency of bacterial infection episodes decreases after the dog has undergone immunotherapy against non-microbial allergens to which the patient is hypersensitive. Unfortunately, there is little published evidence to support this hypothesis and studies are clearly needed to better document such cases.

Evidence for the nature of the relationship between staphylococcal infections and AD exists in four principal areas: (1) increased adherence to and colonization of atopic skin by staphylococci (dogs, man); (2) ‘‘bacterial hypersensitivity’’, i.e. immediate-type hypersensitivity responses to staphylococcal components (dogs, man); (3) the role of staphylococcal exotoxins in serving as superantigens and thereby augmenting the cutaneous

inflammatory response (man); and (4) possible intrinsic immunologic abnormalities of AD and their ability to promote infection (dogs, man).

2.1. Adherence to and colonization of atopic skin by staphylococci

Coagulase-positive staphylococci are regularly and easily cultured from the skin of most AD patients, both human and canine. In one study, *S. aureus* strains were cultured from skin of over 90% of human patients with AD, versus only 5% of normal individuals (Leyden et al., 1974). It is clear that *S. intermedius* is harbored on the skin, haircoat, anal area, or nasal passages of many healthy dogs, at least some of the time; debate continues as to whether it should be considered a resident or transient organism, and there is evidence for both viewpoints (reviewed in Mason et al. (1996)). Factors beyond the mere presence of the bacteria are thus thought to be required for active infection to ensue. In dogs with AD, factors such as self-trauma, corticosteroid therapy, and unspecified ''immunologic abnormalities'' (see below) have been theorized to facilitate staphylococcal infections, though many of these factors are not proven (Scott et al., 2001).

The high prevalence of recurrent staphylococcal infections in dogs with AD can in part be related to greater numbers and adherence of staphylococcal organisms to the skin of such patients. Atopic dogs exhibit significantly higher surface counts of staphylococcal organisms than healthy dogs, and the bacteria are concentrated in the more superficial cornified layers of the epidermis (Mason and Lloyd, 1989). In addition, adherence of staphylococci to keratinocytes of dogs with AD is greater than in healthy dogs or in dogs with primary seborrhea (MacEwan, 1990, 2000). Both numbers of *S. intermedius* organisms and their adherence to keratinocytes appear to decrease with remission of AD in dogs (Harvey and Noble, 1994). The mechanisms by which keratinocyte adherence varies during the course of canine AD have not been studied.

2.2. Staphylococcal hypersensitivity and AD

Two different syndromes of recurrent staphylococcal infection in man have been potentially associated with immediate-type hypersensitivity against staphylococcal antigens. The first, hyperimmunoglobulinemia E (hyper-IgE) syndrome, is characterized by recurrent, often severe and/or deep, staphylococcal infections of the skin and respiratory tract occurring in children (Geha and Leung, 1989). These patients have very high levels of both total serum IgE and IgE specific for staphylococcal antigens (Matter et al., 1986). An analogous syndrome has not been recognized in dogs. The second syndrome occurs in some (but clearly not all) human AD patients with recurrent staphylococcal infections. The latter patients also have detectable antistaphylococcal IgE; however, levels are substantially below those typically reported with hyper-IgE syndrome (Friedman et al., 1985). A subset of human AD patients also was reported to produce IgE antibodies against staphylococcal exotoxins; the toxins could induce IgE-dependent basophil histamine release (Leung et al., 1993). These and other observations led to the hypothesis that, in certain individuals, cutaneous mast cells become sensitized with antistaphylococcal IgE, then degranulate upon exposure to even small amounts of percutaneously-absorbed staphylococcal toxins or other antigens. The inflammatory mediators released would then

inhibit granulocyte chemotaxis or other leukocyte functions, and/or interfere with local defense mechanisms resulting in perpetuation of the infection. These concepts were only theoretical, and despite continuing investigation in this field, additional evidence for IgE-mediated hypersensitivity as a major pathogenetic feature of human AD has not emerged. Authors of one textbook state that ''currently, there is no convincing evidence that staphylococcal allergy plays a clinically significant role in [human] atopic dermatitis'' (Hanifin, 1992). More recently, other mechanisms by which staphylococcal products can exacerbate human AD have been reported (see below); mounting evidence continues to favor such pathways over mere ''bacterial hypersensitivity'' mechanisms.

Evidence for the existence of ''bacterial hypersensitivity'' in dogs is limited. Initial suspicions may have come from clinical observations, in that some dogs with AD and staphylococcal infections present with severely erythematous, spreading, very pruritic lesions that ''suggest'' allergic reactions. Subsequently, certain histologic criteria and positive intradermal test reactions to staphylococcal extracts (the latter not proven to be reaginic antibody-mediated) were used as evidence to support the concept of bacterial hypersensitivity (Scott et al., 1978). More recently, the canine humoral immune response to staphylococcal antigens has been reported in dogs with various forms of recurrent pyoderma (Morales et al., 1994; Shearer and Day, 1997). As might be expected, dogs with prior staphylococcal infection exhibit higher antistaphylococcal IgG levels than healthy dogs, and those with recurrent, deep pyoderma often have the highest levels. However, dogs with recurrent superficial pyoderma secondary either to AD or without recognized underlying causes had significantly higher mean serum levels of antistaphylococcal IgE than healthy dogs or dogs with other forms of pyoderma (Morales et al., 1994). These findings suggest that staphylococcal components could serve as allergens in some dogs, and stimulate an IgE response against the organism. Second, transepidermal penetration of staphylococcal antigens has been demonstrated in dogs, and can be increased with degranulation of mast cells, as would occur in AD (Mason and Lloyd, 1989, 1990). Thus, it has been demonstrated that two necessary components of an immediate-type hypersensitivity reaction (specific reaginic antibody and its corresponding antigen) can be present in canine skin. However, a conclusive, cause-and-effect relationship for a cutaneous immediate-type hypersensitivity reaction against staphylococcal antigens (such as might be demonstrated by passive transfer experiments) has not been established with certainty in dogs.

The concept of ''bacterial hypersensitivity'' and its possible contribution to clinical signs of AD therefore remain hypothetical in dogs. Until the theory, and its diagnostic and therapeutic ramifications (if any) are proven, clinical practices such as intradermal testing with staphylococcal antigens, measurement of antistaphylococcal IgE, and injection of staphylococcal antigen preparations in an attempt to ''desensitize'' the patient are subject to question.

2.3. The role of superantigens and other staphylococcal components in AD

The myriad structural components and secreted products of staphylococci growing on skin may have a variety of effects on the cutaneous inflammatory response. *S. intermedius* bacteria contain a variety of substances (such as protein A, peptidoglycans, and teichoic

acids) that are capable of causing direct inflammatory changes in canine skin (Mason and Lloyd, 1995). Other components, as suggested above, may function as allergens. However, mounting — perhaps overwhelming — evidence demonstrates that toxins released by coagulase-positive staphylococci (the so-called enterotoxins or exotoxins) have undeniable roles in the pathogenesis of human AD. Though bacterial exotoxins can augment hypersensitivity responses in several ways, their function as ''superantigens'' is the subject of most recent research.

In the usual, antigen-specific immune response, a critical initial step in the process is binding of the antigen to MHC class II molecules on antigen presenting cells (APC), and simultaneously to antigen-specific receptors on T-lymphocytes. The APC/T-lymphocyte cross-link formed activates T-lymphocytes specific for the antigen, resulting in an antigen-specific immune response.

In contrast, ''superantigens'' are molecules capable of directly cross-linking the MHC class II molecule with antigen receptors of *any* T-lymphocyte exhibiting a unique region of the β-chain of the T-cell receptor. This phenomenon does not require antigen specificity, and thus induces widespread, polyclonal activation of T-lymphocytes and a broad activation of the immune system. Various exotoxins produced by coagulase-positive staphylococci (e.g. staphylococcal enterotoxins A, B, C, D, and TSST-1) have been clearly established as potent superantigens in the human immune system, and abundant studies in man now support their role in AD (for review, see Skov and Baadsgaard (2000)). Though not all strains of *S. aureus* produce exotoxins, a greater percentage of strains obtained from human patients with AD produce such toxins than strains isolated from healthy controls; also, toxin production is correlated with increased severity of the AD (Zollner et al., 2000).

There are several additional ways by which staphylococcal products could serve as amplifying mechanisms in the cutaneous inflammatory response. Superantigen exotoxins induce cutaneous lymphocyte antigen (CLA) expression on T-lymphocytes, and in addition facilitate expression of the adhesion molecule E-selectin on cutaneous vascular endothelium. The net effect is to facilitate ''homing'' (migration) of activated T-lymphocytes to the site of cutaneous inflammation, thus augmenting the overall inflammatory response (Leung et al., 1995; Strickland et al., 1999). Staphylococcal toxins also directly cause release of cytokines such as TNF-α from human keratinocytes, thereby both contributing to the cutaneous inflammatory response and causing keratinocyte death (Ezepchuk et al., 1996).

Beyond their ability to induce polyclonal T-lymphocyte activation and T-lymphocyte homing, superantigen components of *S. aureus* have been shown in numerous studies to up-regulate the so-called ''type 2'', pro-allergic cytokine response of human lymphocytes, with increased interleukin-4 production, decreased interferon-γ production, and an augmentation of IgE production in response to specific allergens (Campbell and Kemp, 1997; Hofer et al., 1999). Recently, non-superantigen staphylococcal components have been shown to facilitate such responses as well (Jahreis et al., 2000).

To complicate matters further, staphylococcal exotoxins can themselves serve as allergens, and hypersensitivity reactions against the toxins may occur in some human patients. In one study, serum IgE titers against staphylococcal exotoxins were found in approximately half of AD patients with infections, and basophils sensitized with these patients' IgE degranulated upon exposure to the specific toxin to which the patient was sensitive (Leung et al., 1993). More recently, levels of IgE antibodies against

staphylococcal exotoxins in human AD patients have been reported to correlate with the severity of AD symptoms (Bunikowski et al., 1999; Nomura et al., 1999).

Literature consensus is thus overwhelmingly in favor of a strong role for staphylococcal exotoxins in the pathogenesis of human AD. It must be emphasized, however, that virtually none of these observations have been extended to canine AD. The role of *S. intermedius* exotoxin production in the pathogenesis of canine AD and recurrent staphylococcal skin infections is at this time unknown. It has been established that staphylococcal strains isolated from canine skin infections can produce exotoxins. Staphylococcal exotoxins, including enterotoxins A, B, C, D, E, and TSST-1 have been detected in up to 40% of strains of *S. intermedius* isolated from dogs (Adesiyun and Usman, 1983; Hirooka et al., 1988). One study attempted to relate toxin production to lesion type, severity, or other characteristics of staphylococcal infections in dogs, but a direct relationship was not found (Burkett and Frank, 1998). Current evidence thus fails to support a role for these exotoxins in the pathogenesis of recurrent canine infections, however, the evidence is so limited that it is premature to draw any conclusions in the dog. Whether staphylococcal exotoxins or other staphylococcal components serve as superantigens in the canine immune system, and whether they can thereby augment hypersensitivity and clinical signs of canine AD, has not yet been established.

2.4. Immunologic alterations in staphylococcal infection

Limited evidence suggests that dogs with AD present abnormal cell-mediated immune responses, which, in theory, could predispose an animal to cutaneous infection. Nimmo-Wilkie et al. (1991) studied the responses of healthy dogs, dogs with AD, and dogs with non-atopic dermatitis to topical applications of dinitrochlorobenzene and to intradermal injections of mitogens. Dogs with AD exhibited significantly lower responses (as measured by skin thickness) to dinitrochlorobenzene, but significantly higher response to non-specific mitogen injection, than other groups of dogs. Similar results have been reported elsewhere (Umesh et al., 1995). All these results suggest a direct evidence that the cutaneous cell-mediated immune response is altered in dogs with AD. Recent studies in man also suggest alterations in lymphocyte response, and provide evidence that T-lymphocyte apoptosis is a potential mechanism for such immune suppression. The investigators demonstrated suppressed proliferative responses of peripheral blood mononuclear cells from human AD patients to staphylococcal enterotoxin B, but only during times of severe disease exacerbation; the suppressed cell proliferation in severe AD patients was strongly associated with presence of apoptotic T-lymphocytes, possibly induced by the toxin itself (Yoshino et al., 2000).

3. *Malassezia* dermatitis and its relationship to atopy

Lipophilic yeast of the genus *Malassezia* (formerly designated *Pityrosporum*) are another clear contributor to clinical signs of AD in human beings and dogs. The clinical features of yeast dermatitis in dogs are reviewed elsewhere (Akerstedt and Vollset, 1995; Morris, 1999). As with staphylococcal infection, a prominent clinical feature of *Malassezia*

dermatitis is pruritus that sometimes can be severe. *Malassezia* dermatitis is over-represented in certain breeds such as basset hounds, cocker spaniels, and West Highland White Terriers. Perhaps 50% of dogs with *Malassezia* dermatitis are atopic or are affected with other allergic diseases such as food or flea allergy, but primary cornification defects and endocrinopathies also are common concurrently (Bond et al., 1996; Guagere and Prelaud, 1996). Thus, not every dog with *Malassezia* dermatitis is atopic, and not every dog with AD will develop *Malassezia* dermatitis.

3.1. Colonization and strain variation in Malassezia isolates from canine skin

The taxonomy of the genus, prevalence of colonization on dogs, and proposed mechanisms for establishment of colonization on animals has been recently reviewed (Guillot and Bond, 1999). It is well established that *Malassezia pachydermatis* is part of the normal cutaneous microflora of dogs, though differing numbers of yeast occur at different anatomical sites (Bond et al., 1995, 2000; Kennis et al., 1996).

Attempts have been made to relate causation of clinical signs in dogs to strain-related differences. Protein electrophoretic patterns of crude *M. pachydermatis* lysates are similar among isolates (Coutinho et al., 1997). Recent molecular techniques such as random amplification of polymorphic DNA and pulsed-field gel electrophoresis have revealed that clinical isolates of *Malassezia* from man and animals are heterogeneous in their genetic composition. In fact, some strains of *M. pachydermatis* isolated from dogs may be physiologically or phylogenetically closer to *M. furfur*, *M. sympodialis*, or even unrelated to currently-recognized *Malassezia* species, and as many as 80% of isolates taken from dogs may be of mixed yeast species (Raabe et al., 1998; Aizawa et al., 1999; Senczek et al., 1999). The significance of these findings to clinical disease has not been established, although it appears there is no simple or obvious relationship between yeast strain or species and severity of clinical signs.

3.2. Immunologic reactions to yeast and other fungal antigens

Both yeast and dermatophyte fungi can serve as allergens in certain human patients with AD (Nordvall and Johannson, 1990; Wilson et al., 1993). Particularly in the case of AD affecting the head and neck, people with AD often exhibit positive skin-prick tests to yeast antigens, elevated levels of IgE against *Malassezia* antigens, and other evidence of immediate-type hypersensitivity response to the yeast (Kieffer et al., 1990; Rokugo et al., 1990; Rasool et al., 2000; Tengvall et al., 2000). In addition, in human beings, *Malassezia* is capable of modulating cytokine production (triggering a T-helper type 2 response) and could play a role in maintaining IgE-mediated skin inflammation in AD (Kroger et al., 1995; Tengvall et al., 1996). Clinical improvements of human atopic patients has been noted following treatment with systemic antifungal agents (Kolmer et al., 1996).

In dogs, as with staphylococcal infections, there is evidence that under some circumstances an immediate-type hypersensitivity reaction may develop to *Malassezia* antigens. Morris et al. (1998) performed intradermal testing, using *Malassezia* extracts, on a series of dogs with AD, with and without yeast-induced dermatitis. Dogs with AD and *Malassezia* dermatitis exhibited significantly greater test reactions than those with AD and no

Malassezia dermatitis, suggesting development of immediate-type hypersensitivity to yeast antigens in some dogs with AD. Alternatively, this finding could be interpreted as a mere reflection of a normal immune response to *Malassezia* yeast that can be observed in dogs with or without atopy.

In addition, an unpublished preliminary report proposed that dogs with AD exhibit higher levels of serum IgE against *Malassezia* antigens than non-atopic dogs or those with *Malassezia* dermatitis but without AD.[1]

Histopathologic examination of skin biopsy specimens from West Highland White Terrier dogs with *Malassezia* dermatitis and epidermal dysplasia often reveal lymphocytic exocytosis and mast cells aligned in a linear fashion along the dermoepidermal junction (Scott and Miller, 1989). In a different group of dogs, immunohistology of skin biopsy specimens demonstrated numerous CD3-positive T-lymphocytes and immunoglobulin-positive plasma cells (Mauldin et al., 1997). These findings have been put forth as evidence to support presence of cutaneous immediate and/or delayed-type hypersensitivity reaction to superficial yeast organisms. However, it must be kept in mind that such lymphocytic deposition is a finding characteristic of an activation of the skin immune system, and as such cannot be interpreted as very specific for any type of immune reaction.

Bond et al. (1998) compared the *Malassezia*-specific humoral and cellular immune responses of healthy dogs to those with *Malassezia* dermatitis. Serum anti-*Malassezia* IgG antibody titers were significantly higher in dogs with yeast dermatitis, reflecting a humoral immune response to more extensive exposure. Cellular immune response, as measured by lymphocyte blastogenic response to *M. pachydermatis* antigen, was higher in healthy basset hounds than in affected basset hounds or in healthy beagles, suggesting variation in the cellular immune response related both to exposure and to breed. A more recent preliminary report, however, indicated no difference in lymphocyte response to *Malassezia* antigens between healthy dogs and those with AD and/or *Malassezia* dermatitis.[2]

Thus at present, there is only scant, and possibly conflicting, evidence for allergen-specific hypersensitivity reactions to *Malassezia* yeast in dogs. The precise immunologic alterations that occur with yeast dermatitis in the canine species remains to be determined with further study.

The discovery of the importance of staphylococcal superantigens in the pathogenesis of human AD has prompted some investigation as to whether *Malassezia* yeast components could function similarly. Recent studies have failed to demonstrate such superantigen activity and, in fact, have supported the role for an antigen-specific immune response to yeast components (rather than a non-specific, superantigen-mediated mechanism) in man (Johannson et al., 1999). The clinical relevance of other ''non-specific'' immunologic effects triggered by yeast is still undetermined. For example, yeast also contain or secrete a variety of substances that can initiate the complement cascade and trigger an inflammatory response (Belew et al., 1980).

[1] Nuttall, T.J., 1998. *Malassezia*-specific IgE levels in normal and atopic dogs (abstract). In: Proceedings of the 15th Annual Meeting of the European Society of Veterinary Dermatology, Maastricht, The Netherlands, p. 155.

[2] Morris, D.O., Clayton, D.J., Felsburg, P.J., 2001. Response of normal and atopic dogs' peripheral blood mononuclear cells to *Malassezia pachydermatis* extract: a pilot study (abstract). In: Proceedings of the Annual Meeting of American Academy of Veterinary Dermatology/American College Vet Dermatology, Norfolk, VA.

4. Summary and conclusions

Clinical observations, as well as an abundance of experimental observations, strongly support presence of a relationship between cutaneous infections with staphylococci and *Malassezia* yeast and the causation and perpetuation of human AD, and multiple mechanisms for such a relationship. Remarkably similar clinical observations suggest that the same is true for canine AD. However, rigorous studies that establish mechanistically the relationship of recurrent infections to AD are lacking in dogs. These subjects remain fertile ground for further investigation in veterinary dermatology and allergy.

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