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Review: Lymphocytes, cytokines, chemokines and the T-helper 1–T-helper 2 balance in canine atopic dermatitis

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Background – The development of atopic dermatitis (AD) and other cutaneous hypersensitivities involves the activation and differentiation of allergen-specific lymphocytes. Although hypersensitivity is often considered to be a 'T-helper 2-polarized' lymphocyte response, recent evidence suggests that clinical disease is associated with the development of multiple lymphocyte phenotypes.

Objectives – The purpose of this paper is to review recent advances in the understanding of the roles of lymphocytes, cytokines and noncytokine factors in the pathogenesis of canine AD.

Methods – Citation databases, abstracts and proceedings from international meetings published between 2001 and 2013 were reviewed in this update. Where necessary, older articles were included for background information.

Results – The development of canine AD is associated with changes in both cutaneous and circulating lymphocyte populations. These lymphocyte responses are characterized by the production of a complex variety of cytokines, including not only T-helper 2 but also T-helper 1, T-helper 17 and regulatory T-cell responses. In addition, microarray gene expression analysis has enabled the identification of a number of noncytokine factors that appear to be associated with atopic inflammation. These include the calcium-binding protein S100A8, serum amyloid A and a number of protease inhibitors, as well as genes involved in epidermal barrier formation, innate immunity receptors, cell cycle proteins and apoptosis.

Conclusions – The development of AD in dogs is characterized by the development of a delicate balance between a variety of T-cell phenotypes and inflammatory mediators, including cytokines, chemokines and noncytokine factors.

Introduction

The development of cutaneous hypersensitivity reactions (including canine atopic dermatitis; AD) involves a complex interaction between environmental antigens, genetic predisposition and a number of disparate cell types. The ultimate result of these interactions is the activation of both T and B lymphocytes. The function of these lymphocytes is influenced in part by the nature of the inflammatory cytokine and chemokine milieu in which they are activated. Certain cytokines promote the development of a 'T-helper 2 (Th2)' phenotype, which promotes the production of immunoglobulin (Ig) E antibodies and favours the production and recruitment of inflammatory cells typically associated with hypersensitivity, such as eosinophils. The development of new methods to evaluate gene expression (such as gene expression microarrays) has made it clear that canine AD is not completely a Th2 phenomenon. Instead, cutaneous hypersensitivity is the result of a delicate balance between a variety of types of T-cell responses and inflammatory mediators, including not only Th2 cytokines, but also T-helper 1 (Th1), T-helper 17 (Th17) and regulatory cytokines as well as noncytokine factors. The purpose of this paper is to review recent works investigating the roles of

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lymphocytes, cytokines, chemokines and the Th1–Th2 balance in canine AD.

Lymphocytes and cytokines in canine atopic dermatitis

Historical perspective

At the time of the initial 2001 report of the ACVD Task Force on Canine Atopic Dermatitis, much work had already been performed to characterize the immune response in humans with AD, as well as in mouse models of the disease.[1,2] This work had demonstrated a predominantly lymphocyte-driven inflammatory response characterized by the expression of variable proportions of 'Th2' cytokines [e.g. interleukin (IL)-4, IL-5, IL-6, IL-9, IL-10 and IL-13] and 'Th1' cytokines (e.g. IL-2 and interferon-γ (IFNγ).[1–6]

At that time, however, there was still a very limited amount of information regarding the cutaneous immune response in canine AD. As had been found in humans, $CD3^+$ lymphocytes represented a major component of the mononuclear inflammatory infiltrate in atopic dog skin.[7,8] Two studies demonstrated that both $CD4^+$ and $CD8^+$ T lymphocytes could be found in increased numbers in lesional atopic dog skin.[7,8] The dermal lymphocytes mostly exhibited T-cell receptors composed of the αβ chains, while epidermal lymphocytes could express either αβ or γδ T-cell receptors.[7]

Even less information was available regarding the cytokine production by T cells in dogs with AD. Analysis of cutaneous mRNA transcripts demonstrated that IL-4 and IL-5 were more commonly expressed in samples obtained from the lesional skin of dogs with AD relative to normal dogs.[9] In contrast, gene expression of IL-2 and IL-12 was more commonly found in normal dog skin, and expression of IFNγ did not differ significantly between the two groups.[9]

Differences were also demonstrated in the function of immune cells harvested from atopic dogs. Mitogen-pretreated peripheral blood mononuclear cells harvested from atopic dogs were demonstrated to have a decreased ability to suppress (and frequently enhanced) mitogen- or histamine-stimulated cell proliferation of target cells.[10] In contrast, untreated mononuclear cells had an enhanced suppressor effect. The reasons for this apparent paradox in activity were unclear. Possible reasons for this effect included the presence of an enhanced 'baseline' proportion of suppressor cells in atopic patients, leaving fewer cells remaining that were capable of being further induced towards a suppressive phenotype.[10]

Finally, atopic dogs were relatively refractory to experimentally induced hypersensitivity to the contact allergen dinitrochlorobenzene, although they demonstrated greater dermal induration and cellular lymphocytic infiltration (relative to healthy dogs) following intradermal injection of the mitogens concanavalin A and phytohaemagglutinin.[11] These findings suggested a derangement in the cell-mediated immune response in these dogs, possibly in the function of Langerhans cells, which are required for the mediation of contact hypersensitivity but not for the response to intradermal mitogens.[11] Together, these functional studies demonstrated evidence of an abnormal and possibly enhanced nonspecific immunological reactivity in atopic dogs, although the exact nature of this reactivity remained to be determined.

Update on lymphocytes and cytokines in canine atopic dermatitis

Lymphocytes

Lymphocytes are present in low numbers in normal canine skin. Both $CD4^+$ and $CD8^+$ lymphocytes have been demonstrated in the canine epidermis and dermis. Typically, CD8 cells predominate in the epidermis, while CD4 cells predominate in the dermis.[8] Greater numbers of both cell types are present in lesional and nonlesional atopic epidermis as well as in lesional dermis relative to healthy skin.[8] In contrast, nonlesional dermis exhibited an increase in CD8 cells only.[8]

Like other inflammatory cells, lymphocytes can be recruited to inflamed or allergen-challenged skin. Patch testing of experimentally sensitized beagles was associated with epidermal recruitment of lymphocytes, including γδ T cells.[12–14] Similar results were seen after epicutaneous sensitization of naïve Maltese–beagle crossbred dogs, where exposure to the mite allergens resulted in lymphocyte recruitment.[15] Subsequent exposure of the sensitized dogs further increased lymphocyte numbers, suggesting an active role for these cells in the developing hypersensitivity reaction.[15] Intradermal injection of anti-canine IgE antibodies induces antigen-independent cross-linking of mast cell-bound surface IgE, resulting in degranulation and elaboration of inflammatory mediators. This antigen-independent mast cell degranulation has been demonstrated to induce the recruitment of αβ T cells in a fashion similar to that seen after injection of allergen in atopic dogs.[16]

One means by which T lymphocytes may be recruited to atopic skin is via expression of the chemokine receptor CCR4. CCR4 is the receptor for the T-cell chemotactic factor TARC [thymus and activation regulated chemokine; chemokine (C-C motif) ligand 17; CCL17]. TARC has been demonstrated to be upregulated by keratinocytes in lesional canine AD.[17] Circulating $CCR4^+$ T cells are present in greater numbers in spontaneously allergic and experimentally sensitized dogs than in healthy nonallergic dogs.[18] In one study, circulating $CCR4^+$ T cells were variably increased following environmental allergen exposure.[19] Expression of CCR4 mRNA has also been demonstrated in atopic canine skin, where it is probably associated with skin-homing T cells.[20]

There are also changes in circulating T-cell numbers in dogs with AD. In one study, healthy dogs had a circulating $CD4^+$-to-$CD8^+$ ratio of 1.7:1.[21] In contrast, the atopic dogs had a $CD4^+$-to-$CD8^+$ ratio of 2.1:1, while a subset of atopic dogs without pyoderma had an even more exaggerated ratio of 2.97:1. In another study, dogs epicutaneously sensitized by application of mite allergen to tape-stripped skin had a greater percentage of $CD4^+$ cells expressing the activation marker CD30 than dogs that were not tape stripped during sensitization.[22] Increased numbers of $CD25^+$ $CD4^+$ cells have also been reported in experimentally sensitized and challenged

dogs.[19,22] Interpretation of these results can be complicated by the dual nature of CD25, which may be expressed on both activated and regulatory T cells (Treg).[23,24]

Less is known about the behaviour of Treg in the pathogenesis of canine AD, partly because of difficulties associated with conclusive identification of these cells. Certain cell markers have been used as Treg markers, but these may also be expressed by other cell types. For example, CD25 represents the α-subunit of the IL-2 receptor present on the surface of thymus-derived Treg cells.[25] This molecule is also upregulated on activated T cells, and thus the mere presence of CD25 expression cannot be relied upon to identify a Treg phenotype.[26] However, high levels of expression of CD25 ($CD25^{high}$) have been demonstrated to correlate with increased expression of the regulatory transcript and protein FOXP3 in dogs, and thus $CD25^{high}$ cells may be more likely to correspond to Treg.[24] Certain regulatory transcripts (e.g. *FOXP3*) or cytokines [e.g. IL-10, transforming growth factor-β (TGFβ)] may be more reliably associated with Treg cells. However, even this method is not foolproof, because many of these factors also have functions other than simple regulation (e.g. IL-10 may also mediate Th2-type inflammation). In addition, at least six B- and T-regulatory phenotypes expressing different cell markers and transcripts have been identified in humans and rodent models.[27] Simultaneous evaluation of multiple cell markers and/or cellular products may therefore be required for unequivocal identification of regulatory cells. Unfortunately, this is technically challenging and hampered by the lack of appropriate canine-specific reagents. Nonetheless, some information can be obtained from the evaluation of more limited data, as will be discussed under '*Regulatory cytokines*' elsewhere in this article.

Cytokines, chemokines and other proteins in atopic dermatitis

T-Helper 2 cytokines

T-Helper 2-type reactions promote the development of humoral immunity and hypersensitivity responses. Canine and human AD are no longer considered strictly 'Th2-polarized' immune responses, but evidence suggests that Th2-type cytokines are involved in the development and perpetuation of AD, particularly in the early phases and in acute lesions.

Interleukin-4

Interleukin-4 is commonly considered the archetypical Th2 cytokine. It is produced by some T cells, mast cells and possibly basophils.[28,29] Gene and protein expression of IL-4 has been variably found in dogs with AD (Table 1). In one study, IL-4 mRNA transcripts were found in the skin of both healthy and atopic dogs, albeit more frequently in atopic skin.[9] Another study also found overexpression of IL-4 in atopic relative to healthy skin.[30] In contrast, others failed to detect IL-4 gene expression in either atopic or healthy dog skin, or any significant differences in mRNA expression between lesional, nonlesional or healthy skin.[31,32]

Similar inconsistencies can be found in data from experimental models of AD. In one study, IL-4 expression could not be detected at any time before or from 6 to 48 h after intradermal injection of anti-IgE in healthy dog skin.[33] Specific environmental challenge of *Dermatophagoides* spp.-sensitized beagles with mite extract has not been associated with significant increases in IL-4 mRNA.[12,34] Unfortunately, data from nonsensitized control dogs was not presented in the latter studies, precluding comparison to 'normal' dogs.

In another study, peripheral blood mononuclear cells (PBMCs) harvested from dogs experimentally sensitized to Japanese cedar (*Cryptomeria japonica*) showed concentration-dependent expression of IL-4 when cultured with Japanese cedar pollen extract.[35] In contrast, IL-4 mRNA expression was decreased in PBMCs harvested from spontaneously allergic dogs compared with nonallergic dogs.[36] There were, furthermore, no significant changes in IL-4 mRNA in PBMC harvested from dogs after allergen-specific immunotherapy.[36] However, a different study showed that immunotherapy using liposome–nucleic acid immune complexes was associated with both an improvement in clinical condition and a decrease in PBMC IL-4 expression in atopic dogs.[37] Finally, one study evaluating IL-4 protein expression using enzyme-linked immunospot found that experimental sensitization to mite allergens increased IL-4 protein production by canine PBMCs.[38]

Thymic stromal lymphopoietin

Atopic canine skin exhibits increased transcription of thymic stromal lymphopoietin (TSLP) compared with healthy skin (Table 1).[39] This cytokine is produced by keratinocytes and induces maturation and activation of dendritic cells and mast cells.[39] Dendritic cell activation in the presence of TSLP appears to foster a 'Th2-promoting' phenotype. Production of TSLP by primary canine keratinocytes is induced by stimulation with ligands for Toll-like receptor 3 (TLR3) and 4, but not TLR2 or TLR7.[39]

Interleukin-31

Interleukin-31 is a recently described cytokine thought to play an important role in AD and pruritus. Although one study failed to detect IL-31 mRNA expression in the skin of dogs with AD (Table 1), subsequent work demonstrated that stimulation of T cells from mite-sensitized dogs with mite allergen and staphylococcal enterotoxin B induced production of IL-31.[38,40] The IL-31 α-subunit receptor has been identified in canine dorsal root ganglia as well as in the canine DH82 histiocytoma cell line, where it was found to activate both the mitogen-activated protein kinase and Janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling pathways.[38,41] Interleukin-31 could be detected in >50% of serum samples from atopic dogs, but not in dogs with other inflammatory skin diseases or healthy dogs.[41] Finally, administration of IL-31 induced pruritus in laboratory beagles.[41]

Other Th2-related cytokines

Other cytokines and chemokines commonly associated with AD in dogs include IL-13, IL-5, MCP1 (monocyte

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Table 1. Cytokines, chemokines and lymphocyte numbers in spontaneous and experimental canine atopic dermatitis

	Increased in skin	Decreased in skin	Increased in PBMCs, whole blood or serum	Decreased in PBMCs, whole blood or serum	Not found or no difference
Spontaneous disease					
Cytokines and their receptors	IL-1b,[31] IL-2,[30] IL-4,[9,30] IL-5,[9] IL-10,[32] IL-12p40 (nonlesional skin only),[32] IL-12β2r,[42] IL-13,[32] IL-13α2r,[42] IFNγ,[9,30–32] TNFα,[30,31] TSLP[39]	TGFβ[30]	IL-5,[45] IL-31[38,41]	IL-4,[36] IFNγ[36,37,45]	IL-4,[31,32] IL-6,[30] IL-10,[45,51] IL-12p35,[30] IL-12p40,[30] IL-31,[40] TGFβ[32]
Chemokines and their receptors	CCL19,[42] CCL28,[42,52] CCR4,[20] IP-10,[42] MCP1,[42] MCP2,[42] MCP3,[42] TARC[17,31,42]	CCL27 (lesional relative to nonlesional skin)[52]	CCR4[18]		
Cell surface receptors/ specific cell types	CD4,[8] CD8[8]		CD4[21]		CD4/FOXP3[51]
Other immune factors	S100A8,[42,56,57] SAA/SAA3,[56] SERPINB4,[42,56,57] SPINK5,[57] TIMP1[42,56]		S100A8[55]		FOXP3[32]
Experimental models					
Cytokines and their receptors	IL-5,[33] IL-6,[12] IL-12p35,[12] IL-13,[12,33] IL-18,[12] IFNγ[12]		IL-4,[35,38] IL-31[38]	IL-10,[34] IFNγ,[35] TGFβ[34]	IL-2,[12] IL-4,[12,33,34] IL-5,[12] IL-6,[33] IL-8,[12] IL-10,[12] IL-12p40,[12] IL-13,[34] IFNγ,[33] TNFα[12]
Chemokines and their receptors	MCP1,[33] RANTES,[33] TARC[12,33]		CCR4[18,19]	CCR4[19]	RANTES[12]
Cell surface receptors/ specific cell types			CD4/CD25[19,22]		

Cytokines: IL-1b, interleukin-1, a general pro-inflammatory cytokine that activates the acute-phase protein response and enhances recruitment of inflammatory cells; IL-2, interleukin-2, a Th1 cytokine that stimulates T-lymphocyte proliferation; IL-4, interleukin-4, a Th2 cytokine that stimulates IgE production; IL-5, interleukin-5, a Th2 cytokine that stimulates eosinophil proliferation and recruitment; IL-6, interleukin-6, a general pro-inflammatory cytokine that activates the acute-phase protein response and promotes lymphocyte growth; IL-10, interleukin-10, a regulatory T-cell cytokine that has immunosuppressive effects on various inflammatory cells; IL-12, interleukin-12, a cytokine that induces the differentiation of naïve T cells into Th2 cells; IL-12p35, the p35 subunit of IL-12; IL-12p40, the p40 subunit of IL-12; IL-12β2r, the signalling subunit of the IL-12 receptor complex; IL-13, interleukin-13, a Th2 cytokine that stimulates IgE production; IL-13α2r, the high-affinity subunit of the IL-13 receptor complex; IL-18, interleukin-18, a Th1 cytokine that induces production of interferon-γ by T cells and natural killer cells; IL-31, interleukin-31, a Th2 cytokine that promotes the sensation of pruritus; IFNγ, interferon-γ, a Th1 cytokine that activates macrophages and suppresses Th2 responses; TNFα, tumour necrosis factor-α, a general pro-inflammatory cytokine that enhances recruitment of inflammatory cells; TSLP, thymic stromal lymphopoietin, a Th2 cytokine that activates and enhances the release of Th2-promoting cytokines from dendritic cells; TGFβ, transforming growth factor-β, a regulatory T-cell cytokine that has immunosuppressive effects on various inflammatory cells.
Chemokines: CCL19, macrophage inhibitory protein 3b, a chemokine which is chemotactic for dendritic cells and lymphocytes; CCL28, a chemokine which is chemotactic for resting lymphocytes and eosinophils; CCL27, a chemokine which attracts skin-associated memory T cells; CCR4, chemokine receptor 4, the receptor for the chemokines CCL17 (TARC) and CCL22 (MDC); MCP1, CCL2/macrophage chemotactic protein 1, a chemokine which attracts mononuclear cells, basophils and eosinophils; MCP2, CCL8/macrophage chemotactic protein 2, a chemokine which recruits IL-5-producing Th2 lymphocytes; MCP3, CCL7/macrophage chemotactic protein 3, a chemokine which attracts mononuclear cells, basophils and eosinophils; RANTES: CCL5/regulated upon activation, normal T cell expressed and secreted, a chemokine that attracts monocytes, T cells and eosinophils; TARC, CCL17/thymus- and activation-regulated chemokine, a chemokine that attracts T cells.
Cell types: CD4, T lymphocytes that interact with cells bearing major histocompatibility class II (MHC II) molecules and includes Th1, Th2 and some suppressor lymphocytes; CD8, T lymphocytes that interact with cells bearing major histocompatibility class I (MHC I) molecules and includes cytotoxic lymphocytes; CD4/FOXP3, a regulatory T cell that has immunosuppressive effects on various inflammatory cells; CD4/CD25 may refer to either a regulatory T cell or an activated T cell.
Other immune factors: FOXP3, Forkhead box P3, a regulatory protein that has immunosuppressive effects on various inflammatory cells; S100A8, a pro-inflammatory protein that increases chemotaxis in neutrophils; SAA/SAA3, serum amyloid A3, one of several lipoproteins that are increased during inflammation and are part of the acute-phase response; SERPINB4, serine peptidase inhibitor clade B, member 4/squamous cell carcinoma antigen 2, an inhibitor of serine proteases; SPINK5, serine peptidase inhibitor Kazal type 5, an inhibitor of serine proteases that is involved in epidermal and hair morphogenesis; TIMP1, tissue inhibitor of metalloproteinases type 1, an inhibitor of matrix metalloproteinases, which may play a role in tissue fibrosis and remodelling.

chemotactic protein 1; CCL2), RANTES (regulated upon activation, normal T cell expressed and secreted; CCL5), MCP3 (monocyte chemotactic protein 3; CCL7), TARC and TSLP.[9,17,32,39,42] Interleukin-10 has also been associated with allergic diseases; however, this cytokine may mediate either Th2-type inflammation or act as a regulatory cytokine.[32,43] Studies investigating the expression of these mediators are summarized in Table 1.

T-Helper 1 cytokines

T-Helper 1 cytokines induce cell-mediated immune responses and, to a certain extent, antagonize Th2 responses. Atopic diseases were originally considered to involve a Th2–Th1 imbalance, but it is now known that Th2 cytokine profiles predominate only during sensitization and in acute lesions of AD. T-Helper 1 responses contribute to the development of clinical disease, and may predominate in chronic lesions.

Interferon-γ

Interferon-γ is considered the 'canonical' Th1 cytokine, much as IL-4 is for Th2 immune responses.[44] Interferon-γ mRNA transcripts have been detected in skin biopsies of dogs with spontaneous AD, most frequently from chronic, lichenified lesional skin.[9] Levels in canine lesional atopic skin are higher than in nonlesional or healthy skin.[30–32]

Intradermal injection of anti-IgE did not induce the transcription of IFNγ in the skin of healthy nonallergic beagles, suggesting that it is not involved in immediate-phase responses.[33] In contrast, environmental allergen challenge of mite-sensitized beagles induced increased transcription of IFNγ as well as IL-12p35 (although not IL-12p40), primarily during the later stages of inflammation.[12] Peripheral blood mononuclear cells from dogs with spontaneous or experimentally induced AD have lower levels of spontaneous and *Dermatophagoides* spp.-induced IFNγ expression than PBMCs from control dogs, suggesting a Th2 PBMC phenotype.[35,36,45] Expression of IFNγ in mite-sensitized dogs with AD increased after allergen-specific immunotherapy, suggesting that immunotherapy was associated with induction of Th1 cells.[36] In contrast, a separate study evaluating the response to allergen-specific immunotherapy failed to demonstrate any increase in IFNγ expression even in dogs with a good clinical response.[37]

Interleukin-12

Interleukin-12 is composed of two subunits, IL-12p35 and ILp40, which combine to form IL-12p70 (sometimes referred to as IL-12p75).[46,47] In humans, the IL-12p35 subunit can also be incorporated into the heterodimeric cytokine IL-35, but this cytokine has not yet been identified in the dog.[46] Messenger RNA for IL-12p35 appears to be transcribed constitutively [48], but the protein is secreted only in the heterodimer form.[49] This discrepancy complicates interpretation of gene transcription data for this subunit, because mRNA expression does not necessarily correlate with protein secretion. The IL-12p40 subunit is not constitutively expressed or secreted and is sometimes considered a more 'specific' indicator of Th1-type inflammation.[49] However, this subunit is also shared by the heterodimeric cytokine IL-23.[46] Interleukin-23 may play a greater role in Th17 immune responses, which are associated with mucosal immunity and may be involved in autoimmune diseases.

Interleukin-12 expression is more varied in atopic dogs than is IFNγ expression (Table 1). In one study, neither IL-12 subunit was differentially expressed in the skin of atopic dogs, while another study found that nonlesional atopic dog skin expressed more IL-12p40 than lesional and healthy skin.[30,32] A gene expression microarray study found that canine lesional skin exhibited differential expression of a number of Th1-associated inflammatory factors, including the IL-12 receptor β2-subunit and CXCL10 (interferon-γ-inducible protein 10; IP-10), which is chemotactic for monocytes and T cells.[42]

Ratios of Th1 to Th2

Some immunologists feel that it is more appropriate to evaluate relative levels of expression of Th1- and Th2-type cytokines.[36] Both Th1 and Th2 responses are involved in AD, and truly 'polarized' Th2 or Th1 cytokine profiles are rarely identified. In one study, only 25% of the atopic dog samples examined exhibited a clear 'Th2' cytokine profile, and only 25% of healthy skin samples exhibited a clear 'Th1' profile.[9] Another study investigating expression levels of multiple cytokines and transcription factors also failed to identify a clearly polarized profile.[32]

One study followed the cytokine profile of PBMCs from spontaneously mite-sensitized dogs after culture with mite allergens. Initially, transcription of both IL-4 and IFNγ was decreased relative to PBMCs from healthy control dogs.[36] Interleukin-4 transcription did not change following allergen-specific immunotherapy, but IFNγ transcription increased, leading the authors to suggest that evaluation of cytokine ratios may provide a more effective estimate of immune response than absolute expression values.[36] Another study found a decreased ratio of IFNγ to IL-4 protein expression in dogs following experimental sensitization to *Dermatophagoides farinae* extract.[38] However, this decreased ratio became less apparent upon repeated rechallenge, because IFNγ protein expression increased over time.[38]

Regulatory cytokines

As mentioned elsewhere in this review, evaluation of the role of regulatory mediators (such as IL-10, TGFβ and FOXP3) can be challenging, because some putative immunosuppressive factors may also be pro-inflammatory. Interleukin-10, in particular, can either mediate Th2-type inflammation or downregulate immune responses. Interpreting the role of these 'regulatory mediators' can be complicated further when expression is increased in inflamed skin. It is known that regulatory mechanisms can be overwhelmed in certain circumstances, e.g. in the presence of high levels of IL-6.[50] For this reason, it is often difficult to determine whether these multifunctional cytokines are actively contributing to inflammation or are attempting (and failing) to control it.

Transforming growth factor-β

Decreased TGFβ expression has been reported in atopic compared with healthy canine skin, although this has not

been demonstrated consistently.[30,32] In addition, expression of TGFβ was found to be decreased after environmental allergen challenge in experimentally sensitized dogs.[34]

Interleukin-10
Elevated IL-10 expression has been demonstrated in canine lesional atopic skin.[32] In contrast, there was no difference in spontaneous expression of IL-10 in circulating PBMCs from atopic and healthy dogs.[45] Similar conflicting results have been seen in experimental models of canine AD. In one study, cutaneous mRNA expression of IL-10 did not change significantly following environmental allergen challenge of mite-sensitized beagles.[12] However, a second study demonstrated decreased IL-10 expression in PBMCs after environmental allergen challenge in sensitized beagles.[34] As described above, the ability of IL-10 to promote either Th2 or regulatory immune responses may be at least a partial explanation for these apparently contradictory results.

Forkhead box P3
To date, very little work has been performed to evaluate the role of FOXP3 in the pathogenesis of canine atopic dermatitis. In one study, a trend towards increased FOXP3 expression was demonstrated in canine lesional atopic skin, although this change was not statistically significant.[32] Allergen-specific immunotherapy has been associated with an increase in circulating $CD4^+FOXP3^+$ Treg cells, whereas pre-immunotherapy Treg numbers did not differ from those of healthy control dogs.[51] The presence of normal or even slightly supranormal FOXP3 expression suggests that clinical disease in canine AD may be associated with a functionally decreased regulatory capability of cells expressing this transcript or protein.

Regulatory cytokines and immunotherapy
Allergen-specific immunotherapy may induce tolerance, possibly through IL-10-associated mechanisms. In one study, baseline serum IL-10 levels were similar in atopic dogs and healthy dogs, but increased in atopic dogs following successful immunotherapy. Dogs with poor responses to immunotherapy, in contrast, had significantly lower IL-10 levels.[51] However, in another study the mRNA expression of IL-10 did not change significantly following immunotherapy with liposome–nucleic acid complexes.[37] In a third study, the number of circulating $CD4^+FOXP3^+$ cells was found to increase following immunotherapy.[51] Taken together, these results suggest that in some patients, allergen-specific immunotherapy may be associated with an increase in regulatory T-cell populations.

Other disease-related cytokines and chemokines
Numerous other cytokines can be present in atopic and nonatopic inflammation. These include IL-1, IL-2, IL-6, TNFα, IL-8 and IL-18.[12,30,31] These cytokines are often multifunctional and may mediate nonspecific or nonallergic inflammation. It is therefore not entirely surprising that expression values for these cytokines are often variable or directly contradictory (Table 1).

Several studies have evaluated the role of chemotactic cytokines in canine AD. Lesional atopic skin had decreased mRNA expression of the cutaneous T-cell-homing chemokine CCL27 compared with nonlesional skin, whereas expression of CCL28 (which is chemotactic for resting $CD4^+$ and $CD8^+$ cells as well as eosinophils) was increased.[42,52] Both lesional and nonlesional atopic skin constitutively expressed CCL4, CCL19, CCL20, CCL21 and CCL24 (eosinophil chemotactic protein 2).[52] These factors are chemotactic for lymphocytes and eosinophils and, to a lesser extent, neutrophils and mononuclear cells. Unfortunately, no data from nonatopic canine skin were provided for comparison. A gene expression microarray analysis also demonstrated increased expression of CCL19 (macrophage inhibitory protein 3b; which is chemotactic for dendritic cells and lymphocytes) in atopic relative to healthy skin.[42]

Noncytokine proteins in atopic dermatitis
Several noncytokine proteins have also been associated with AD, including the calcium-binding protein S100A8. This is a pro-inflammatory protein that has been shown to induce chemotaxis in canine neutrophils. Release is stimulated by TNFα, the levels of which are correlated with clinical severity.[53] The S100 gene is also located on the epidermal differentiation complex with profilaggrin, loricrin and involucrin, which are essential for keratinocyte and epidermal barrier differentiation.[54] Serum levels of S100A8 correlate with Canine Atopic Dermatitis Extent and Severity Index scores in atopic dogs, and increased gene expression was demonstrated in lesional atopic skin.[42,55–57] Finally, although a canine genome-wide linkage study failed to detect any chromosomal regions significantly linked to AD, the authors were unable to exclude linkage to a 56 cM region on CFA (canine chromosome) 7, which contains the gene for S100A8.[58]

Differential expression of serine peptidase inhibitor clade B, member 4/squamous cell carcinoma antigen 2 (SERPINB4/SCCA2), serum amyloid A (SAA) and/or its variant SAA3, tissue inhibitor of metalloproteinases 1 (TIMP1) and serine peptidase inhibitor, Kazal type 5 (SPINK5) have also been demonstrated in canine lesional atopic skin, although these changes were not always statistically significant relative to nonlesional or control skin.[42,56,57] Many of these factors may be expressed nonspecifically in other inflammatory conditions. SERPINB4 appears to play a role in tumour metastasis.[59,60] Serum amyloid A belongs to a group of apolipoproteins that are highly expressed in response to inflammation and tissue injury and are considered part of the acute-phase response.[61] TIMPs are inhibitors of matrix metalloproteinases, which are involved in the degradation of extracellular matrix proteins and may play a role in fibrosis and tissue remodelling of chronic lesions.[62,63] Finally, SPINK5 is a serine protease inhibitor that is involved in skin and hair morphogenesis. Mutations of SPINK5 in humans have been associated with Netherton syndrome, which is characterized by ichthyosis and AD.[64] Although none of these factors is specific for allergic disease, it is interesting to note that three of these are proteinase inhibitors. It remains to be seen whether their upregulation is in response to release of endogenous or exogenous proteo-

lytic factors (in which case they might be expected to be attempting to downregulate cutaneous inflammation) or if they are actively participating in the inflammatory response in some other fashion.

Microarrays/gene expression

Advances in gene expression microarray analysis technology have permitted the simultaneous analysis of anything from dozens to thousands of mRNA transcripts from relatively small sample sizes. Expression microarrays may evaluate the entire genome or they may be custom designed to focus on more limited gene sets. Two studies used gene expression microarrays to evaluate patterns of gene expression in canine AD.[42,56] Although a detailed discussion of the results of these studies is beyond the scope of this manuscript, a summary of their findings is presented here.

The first study evaluated gene expression in canine lesional atopic, nonlesional atopic and healthy skin.[56] RNA from these samples was hybridized to a canine-specific Agilent 22K oligonucleotide array (Agilent Technologies, Palo Alto, CA, USA), which evaluated the transcription of ~22,000 genes. Of the 22,000 genes, 54 were significantly differentially expressed in atopic skin relative to healthy skin. Sixteen of the 54 were differentially expressed in atopic skin relative to healthy skin, 12 were differentially expressed only in lesional skin and 26 were differentially expressed only in nonlesional skin.

Genes that were differentially transcribed in both lesional and nonlesional skin of atopic dogs could be grouped by the following general functions: inflammation/immunology; cell cycle/apoptosis/repair/lesion formation; translational control; transport/regulation; barrier formation; and miscellaneous genes. Most of these genes were upregulated relative to healthy skin and included members of the GOLGA4 subfamily, which are involved in ceramide transport to the cell surface.[56]

Genes differentially transcribed only in lesional atopic skin included those involved in the following functions: inflammation/immunology; cell cycle/apoptosis/repair/lesion formation; transport/regulation; barrier formation; and miscellaneous. With a single exception (cell death-inducing DNA fragmentation factor A-like effector c; CIDE-3 or CIDEC), all of these genes were upregulated relative to healthy skin. These included S100A8, SAA and SAA3, SERPINB4/SCCA2 and TIMP1.[56]

Genes differentially transcribed only in nonlesional skin of atopic dogs had the following functions: inflammation/immunology; cell cycle/apoptosis/repair/lesion formation; transport/regulation; barrier formation; transcription factors; and miscellaneous. With few exceptions, these genes were downregulated relative to their expression in healthy skin.[56]

A second whole-genome microarray study also evaluated lesional atopic, nonlesional atopic and healthy skin[42] using the Affymetrix canine 2.0 genome. This array evaluated the transcription of ~18,000 mRNA/expressed sequence tag (EST) transcripts and a further 20,000 predicted genes. In this study, 1070 transcripts were differentially expressed in acute lesional atopic skin compared with healthy skin, 312 transcripts were differentially expressed in nonlesional skin compared with healthy skin and 764 transcripts were differentially transcribed in acute lesional skin relative to nonlesional skin. The transcription data sets from the atopic dogs shared common network pathways with human AD and asthmatic chronic rhinosinusitis with nasal polyps (aCRSwNP).

Genes that were differentially expressed in acute lesional skin had the following functions: genes corresponding to alternatively activated (IL-4- or IL-13-induced) monocytes; anti-apoptotic genes; cell adhesion; other chemokines, interleukins and receptors; collagens; complement-associated genes; eosinophil-associated G-protein-coupled receptors; other G-protein-coupled receptors and associated signalling molecules; members of the IL-1 family, innate immunity receptors and Nod-like receptors; interferon-induced genes; keratins; leucocyte immunoglobulin-like receptors; lipid-associated genes; genes involved in L-tryptophan metabolism; lymphocyte antigens; mast cell-associated genes; proteases and protease inhibitors; S100 family proteins; solute carriers; and transcription factors and zinc finger proteins.[42]

Some differences between these two studies are to be expected given that different microarrays (each with its own proprietary set of gene sequences in addition to more commonly evaluated consensus genes), different case populations and somewhat different analysis algorithms were used. Despite this, the overall concordance between the two array studies is striking. Both were able to identify individual genes of interest as well as groupings of relevant genes. As such, these results may be 'springboards' towards more focused evaluation of novel gene targets.

Conclusion

In summary, recent work has challenged the notion of AD as a purely 'Th2-polarized' phenomenon. Markedly different gene expression profiles for Th1, Th2, regulatory and 'nonspecific' cytokines are detected during different phases of the disease. The development of more advanced methods of gene and protein detection has identified 'new' relevant inflammatory mediators and non-cytokine factors. Furthermore, the advent of gene expression microarray technology provides the potential to define inflammatory network pathways in canine AD further and identify new targets for further study.

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Résumé

Contexte – Le développement de la dermatite atopique (AD) et des autres hypersensibilités cutanées implique la différenciation et l'activation des lymphocytes spécifiques d'allergènes. Bien que l'hypersensibilité soit souvent considérée comme étant une réponse lymphocytaire polarisée T-helper-2, une preuve récente suggère que la maladie clinique est associée au développement de phénotypes lymphocytaires multiples.
Objectifs – Le but de cet article est de revoir les données récentes dans la compréhension des rôles des lymphocytes, des cytokines et des facteurs non-cytokiniques dans la pathogénie de la dermatite atopique canine.
Méthodes – Les bases de données, les résumés et les proceedings des congrès internationaux publiés entre 2001 et 2013 ont été revus dans cette mise à jour. Lorsque nécessaire, des articles plus anciens étaient inclus pour plus d'information.
Résultats – Le développement de l'AD canine est associé à des changements des populations lymphocytaires cutanées et circulantes. Ces réponses lymphocytaires sont caractérisées par la production d'une variété complexe de cytokines n'incluant pas seulement les T-helper-2 mais aussi les T-helper-1, T-helper-17 et les cellules T régulatrices. En outre, l'analyse de l'expression des gènes par puces a permis d'identifier un certain nombre de facteurs non-cytokiniques semblant être associé avec l'inflammation atopique. Ceci inclut la protéine S100A8, la serum amyloïde A et un certain nombre d'inhibiteurs de protéases ainsi que des gènes impliqués dans la formation de la barrière épidermique, les récepteurs de l'immunité innée, les protéines du cycle cellulaire et l'apoptose.
Conclusions – Le développement de l'AD du chien est caractérisé par le développement d'une balance délicate entre une variété de phénotypes cellulaires et de médiateurs de l'inflammation, comprenant des cytokines, chémokines et des facteurs non-cytokiniques.

Resumen

Introducción – el desarrollo de la dermatitis atópica (AD) y de otros procesos de hipersensibilidad cutánea implica la activación y diferenciación de linfocitos específicos de alérgeno. Aunque la hipersensibilidad se

considera a menudo polarizada a una respuesta de linfocitos ayudantes de tipo dos, hallazgos recientes sugieren que la enfermedad clínica está asociada con el desarrollo de fenotipos múltiples de linfocitos.
Hipótesis/Objetivos – el objetivo de este artículo fue revisar avances recientes en la comprensión del papel de linfocitos, citoquina y factores no relacionados con citoquinas en la patogenia de la dermatitis atópica canina.
Métodos – se revisaron para este artículo citas en base de datos, resúmenes y manuales de reuniones internacionales publicadas entre los años 2001 a 2013. Cuando fue necesario, se incluyeron citas de artículos más antiguos como información de soporte
Resultados – el desarrollo de la dermatitis atópica canina está asociado con cambios en las poblaciones de linfocitos cutáneos y circulantes. Esta respuesta linfocitaria está caracterizadas por la producción una variedad compleja de citoquinas, incluyendo no sólo citoquinas de tipo T2 ayudante, sino también de tipo T1, T 17 ayudante, y respuestas regulatorias de linfocitos T. Además, mediante análisis de la expresión génica en micro matrices se han identificado un número de factores no relacionadas con citoquinas que están asociados con la inflamación durante la atopia. Estos factores incluyen la proteína S100A8 de unión al calcio, amiloide sérico de tipo A, y un número de inhibidores de proteasas, así como genes implicados en la formación de la barrera de la piel, receptores de inmunidad innata, proteínas del ciclo celular y de apoptosis.
Conclusiones e importancia clínica – el desarrollo de la dermatitis atópica en perros se caracteriza por la aparición de un balance delicado entre una variedad de fenotipos de linfocitos T y de mediadores de inflamación, incluyendo citoquinas, quimioquinas y factores no relacionados con citoquinas.

Zusammenfassung

Hintergrund – Zur Entwicklung der atopischen Dermatitis (AD) und anderer kutaner Hypersensibilitäten gehört die Aktivierung und Differenzierung der allergen-spezifischen Lymphozyten. Obwohl die Hypersensibilität oft als „T-Helfer 2-polarisierte" Lymphozytenantwort verstanden wird, weist jüngste Evidenz darauf hin, dass die klinische Erkrankung mit der Entwicklung multipler Lymphozyten Phänotypen einhergeht.
Ziele – Das Ziel dieser Publikation ist es jüngste Fortschritte beim Verständnis der Rollen der Lymphozyten, der Zytokine und der nicht-Zytokinen Faktoren bei der Pathogenese der AD des Hundes zu reviewen.
Methoden – Die Zitationsdatenbanken, Abstracts und Proceedings von internationalen Treffen, die zwischen 2001 und 2013 publiziert worden waren, wurden reviewed. Wo es nötig war, wurden ältere Artikel für die Hintergrundinformation inkludiert.
Ergebnisse – Die Entstehung der caninen AD hängt sowohl mit Veränderungen der kutanen wie auch der zirkulierenden Lymphozytenpopulationen zusammen. Diese Lymphozytenpopulationen werden charakterisiert durch die Produktion einer komplexen Variation an Zytokinen, welche nicht nur aus T-Helfer 2, sondern auch aus T-Helfer 1, T-Helfer 17 und regulatorischen T-Zell Reaktionen bestanden. Zusätzlich hat es die Mikroarray Genexprimierungsanalyse möglich gemacht, eine Anzahl an nicht-Zytokinen Faktoren zu identifizieren, die bei der atopischen Entzündung vorkommen. Zu diesen Faktoren zählen das Kalzium-bindende Protein S100A8, Serum Amyloid A und einige Protease Inhibitoren, sowie Gene, die bei der Bildung der epidermalen Barriere beteiligt waren; angeborene Immunrezeptoren, Zellzyklusproteine und die Apoptose.
Schlussfolgerungen – Die Entstehung der AD der Hunde wird durch die Entwicklung einer delikaten Balance zwischen einer Vielzahl von T-Zell Phänotypen und Entzündungsmediatoren, wie Zytokine, Chemokine und nicht-Zytokine Faktoren charakterisiert.

摘要

背景 — 异位性皮炎(AD)和其他皮肤过敏症的发生,与抗原特异性淋巴细胞的激活和分化有关。过敏症经常被认为是“T辅助细胞2极化”的反应,但是最近有证据显示这种疾病是多重淋巴细胞表型出现的结果。
目的 — 本文目的是回顾近期淋巴细胞、细胞因子和非细胞因子,理解其对犬AD发病机制所起作用的最新进展。
方法 — 引用发表于2001至2013年之间国际会议发表的数据、摘要和会议记录,基于这些更新进行综述。
结果 — 犬AD的发病与皮肤和循环淋巴细胞群的变化有关。这些淋巴细胞的反应以产生复合多样的细胞因子为特征,其中不仅包括T辅助细胞2,也有T辅助细胞1、17,以及调解T细胞反应。另外,微阵列基因表达分析能够鉴别许多非细胞因子,这些细胞因子都显现与异位性炎症有关。它们包括钙连蛋白S100A8、血清淀粉样A和一些蛋白酶抑制剂,以及表皮屏障形成、先天免疫受体、细胞循环蛋白和凋亡基因。
结论 — 犬的AD发生特征,是在多种T细胞表型和炎性介质,包括细胞因子、趋化因子和非细胞因子之间,发生了微妙的平衡。

要約

背景 — アトピー性皮膚炎(AD)およびその他の皮膚過敏症の発生はアレルゲン特的リンパ球の活性化や分化に関与している。過敏症はしばしば’Tヘルパー2極性’リンパ球反応と考えられているが、新しいエビデンスによると臨床疾患が複数のリンパ球表現型の発達と関係していると示唆されている。
目的 — この論文の目的はイヌADの病因におけるリンパ球、サイトカイン、非サイトカイン因子の役割の理解についての最近の進歩を総括することである。

方法 — 文献引用データベース、2001年から2013年の間に発表された国際会議での要約や抄録をこの最新情報で再検討した。必要な場合は、背景にある情報のための古い論文を含んでいる。
結果 — イヌADの発生には皮膚と循環するリンパ球の集団の両方の変化が関係している。それらのリンパ球反応は、T−ヘルパー2だけでなくT−ヘルパー1、T−ヘルパー17ならびに制御性T細胞反応を含む、複雑で多様なサイトカインの産生により特徴付けられている。さらに、マイクロアレイ遺伝子発現解析によりアトピー性炎症と関連すると思われる非サイトカイン因子の数を特定することが可能となった。それらにはカルシウム結合タンパクS100A8、血清アミロイド、ならびにプロテアーゼ阻害剤の数だけでなく、表皮バリア形成、自然免疫受容体、細胞周期プロテインおよびアポトーシスに関連した遺伝子が含まれている。
結論 — イヌにおけるADの発生は多様なT細胞表現型およびサイトカイン、ケモカインならびに非サイトカイン因子を含む炎症性メディエーターの間の繊細なバランスの発達によって特徴付けられる。

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