

Cytokines and chemokines orchestrate atopic skin inflammation

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Atopic dermatitis (AD) is a common pruritic and chronically relapsing inflammatory skin disease. The pathophysiology of AD includes disturbed skin barrier functions, frequent allergic responses against allergens, defects in the antimicrobial immune defense, and a genetic predisposition. In this review we summarize advances in our understanding of the complex interdependent network of members of the rapidly growing protein superfamilies of cytokines and chemokines that lead to the development of AD. (*J Allergy Clin Immunol* 2006;118:178-89.)

Key words: Atopic dermatitis, immunity, pruritus, lymphocyte, trafficking

Atopic dermatitis (AD) is a common pruritic and chronically relapsing inflammatory skin disease with a steadily increasing prevalence. Endogenous, as well as exogenous, factors play a role in the manifestation of this disease, which has a serious effect on patients, their families, and socioeconomic aspects of life. The pathophysiology of AD includes a disturbed skin barrier function (for review, see article by Cork et al¹ in this issue of the *Journal*), frequent allergic responses against allergens, defects in the antimicrobial immune defense, and a genetic predisposition. Recent studies provide insights into the underlying immunologic mechanisms, suggesting an amplification cycle of atopic skin inflammation. In this

Abbreviations used

AD: Atopic dermatitis
CD40L: CD40 ligand
CLA: Cutaneous lymphocyte-associated antigen
DC: Dendritic cell
IL-31RA: IL-31 receptor A
LC: Langerhans cell
OSMR: Oncostatin M receptor
TSLP: Thymic stromal lymphopoietin

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OVERVIEW OF ATOPIC SKIN IMMUNOLOGY

Clinical observations in AD combined with the results of recent scientific studies suggest an amplification cycle of atopic skin inflammation (Fig 1). This cycle might start with pruritus, representing a prominent symptom of AD.² Patients scratch and induce mechanical injury, resulting in proinflammatory cytokine and chemokine production. Subsequently, chemokines, in concert with an array of adhesion molecules, direct the recruitment of pathogenic leukocytes to the skin.³⁻⁶ Within the skin, distinct leukocyte subsets can become activated through different pathways: (1) epithelial cell-derived cytokines (eg, thymic stromal lymphopoietin [TSLP]) instruct dendritic cells (DCs) to induce T_H2 cell differentiation⁷⁻¹¹; (2) memory T cells encounter their specific antigen-allergen (eg, Der p 2 from house dust mites) or bacterial superantigens (eg, staphylococcal enterotoxins)⁴; (3) DCs bind antigen-specific IgE complexes, capture antigen, and show enhanced antigen presentation capabilities⁴; (4) antigen-specific IgE complexes induce Fcε receptor aggregation and activate mast cells⁴; and (5) viruses, fungi, and bacteria take advantage of a reduced level of antimicrobial peptides present in AD skin, colonize inflamed skin, and release proinflammatory products, resulting in the modulation and amplification of leukocyte activation.^{12,13} As a common feature, leukocyte activation results in the release of inflammatory mediators, including effector cytokines (IL-31) and

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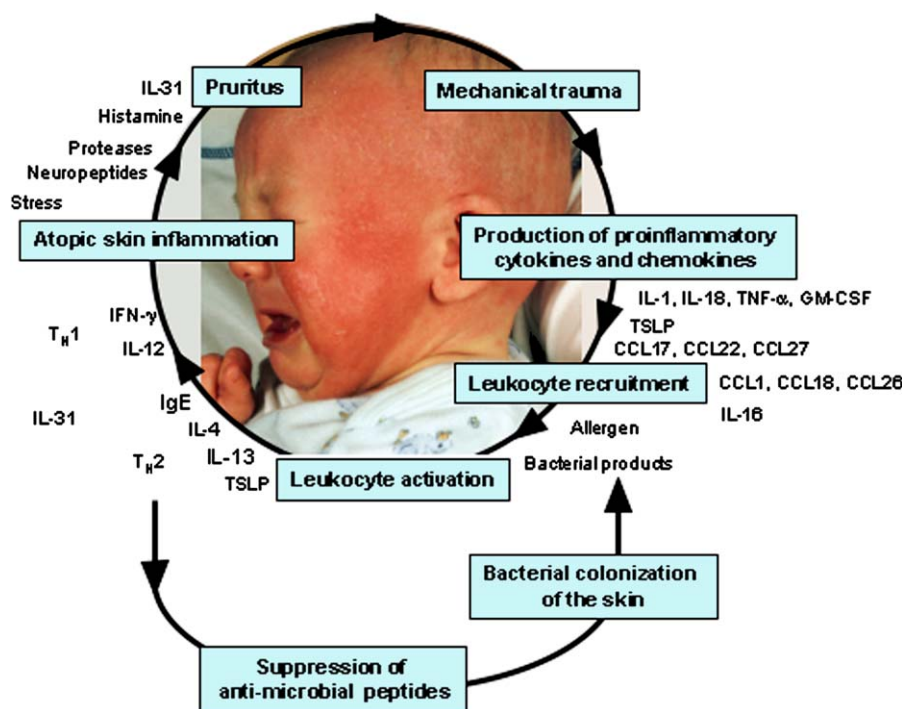


FIG 1. Amplification cycle of atopic skin inflammation. Pruritus represents a prominent symptom of AD. Patients scratch and induce mechanical injury, resulting in proinflammatory cytokine (IL-1, IL-18, TNF- α , and GM-CSF) and chemokine (CCL27) production. Subsequently, chemokines (CCL1, CCL11, CCL18, CCL22, CCL26, and CCL27), in concert with an array of adhesion molecules, direct the recruitment of leukocytes to the skin. Within the skin, distinct leukocyte subsets can be activated through different pathways: (1) memory T cells encounter their specific antigen-allergen or bacterial superantigens; (2) epithelial cell-derived cytokines (eg, TSLP) instruct DCs to induce T_H2 cell differentiation; (3) DCs bind antigen-specific IgE complexes, capture antigen, and show enhanced antigen presentation capabilities; (4) antigen-specific IgE complexes induce Fc ϵ receptor aggregation and activate mast cells; and (5) effector cytokines (IL-4 and IL-13) of activated T_H2 cells suppress the production of antimicrobial peptides (human β -defensin 2 and 3). Viruses, fungi, and bacteria (eg, *S aureus*) take advantage of the decreased amount of antimicrobial peptides present in the skin of atopic individuals, colonize the skin, and release proinflammatory products (eg, superantigens, proteoglycan, and lipoteichoic acid), resulting in the modulation and amplification of leukocyte activation. As a common feature, leukocyte activation results in the release of inflammatory mediators, including effector cytokines (IL-31) and proteases (trypsinase), which, along with stress-induced neuropeptides, perpetuate pruritic signals.

proteases (trypsinase), which, together with stress-induced neuropeptides, perpetuate pruritic signals.^{2,14-16} Taken together, these amplifying processes sustain inflammatory responses within the skin and lead to the development of an AD phenotype.

PRODUCTION OF PRIMARY PROINFLAMMATORY CYTOKINES

Little is known about the very early events initiating atopic skin inflammation. However, the production of primary proinflammatory cytokines after mechanical trauma and skin barrier disruption is a well-accepted scenario. Clinically, patients with AD show significantly lower itch thresholds and do not have skin lesions unless they scratch. Furthermore, experimental studies in mice support that the itch-scratch cycle is important for the development of dermatitis.¹⁷ Numerous studies showed that mechanical trauma and skin barrier disruption after tape stripping of

the skin results in the rapid and marked upregulation of IL-1 α , IL-1 β , TNF- α , and GM-CSF.^{18,19} These primary proinflammatory cytokines in turn induce the production of chemoattractants and adhesion molecules (eg, intercellular adhesion molecule 1), supporting the recruitment, proliferation, and survival of leukocytes at cutaneous sites. Next to mechanical trauma, the staphylococcal product peptidoglycan and histamine have been suggested to contribute to the increased expression of GM-CSF in keratinocytes within the lesional skin of patients with AD.^{20,21} Hence GM-CSF is regulated under the control of innate and adaptive immune pathways and might support the survival and retention of DC subsets at sites of atopic skin inflammation.

Recently, a member of the rapidly expanding IL-1 superfamily, IL-18, has been shown to participate in the development of a relapsing dermatitis with mastocytosis, T_H2 cytokine accumulation, and systemic increase of IgE and histamine levels.²² Various cell types, including macrophages and keratinocytes, store IL-18 as a biologically inactive precursor. Caspase-1 or caspase-like

enzymes cleave pro-IL-18 and release the active form after stimulation by danger signals. Simultaneous skin-specific expression of IL-18 and caspase-1 leads to this atopy-like dermatitis.²² Interestingly, the dermatitis phenotype is independent of signal transducer and activator of transcription 6–signaling pathways but critically dependent on the presence of active IL-18.²² Therefore Konishi et al²² suggest that IL-18 induces IgE-independent allergic disorders and mediates an innate-type of allergic response. In contrast to these findings, Higa et al²³ could not demonstrate a role for IL-18 in the development of AD-like skin lesions in NC/Nga mice. Although IL-18 serum levels were significantly increased before and during the development in NC/Nga mice, administration of a neutralizing antibody failed to inhibit the onset and development of dermatitis and IgE level increase. The treatment, rather, tended to lead to an exacerbation of dermatitis and scratching behavior.²³ Keeping in mind that genetic approaches, intervention studies with neutralizing antibodies, and murine models for human diseases have their own limitations, the findings of a recent study by Novak et al²⁴ support a role for IL-18 in the pathogenesis of AD. Single nucleotide polymorphisms in exon 1 and the promoter region of the *IL18* gene were significantly associated with an AD phenotype. These associations were independent of the concomitant manifestation of allergic rhinitis or asthma. Furthermore, the amount of IL-18 in the supernatants of PBMCs of patients with AD stimulated with staphylococcal enterotoxin B was significantly higher than that in healthy control subjects. In parallel, the amount of active IL-18 in the sera of patients with AD was enhanced at the exacerbation of their disease. These observations suggest that single nucleotide polymorphisms in the *IL18* gene might be involved in the development of AD by contributing to a functional dysregulation of the IL-18 production *in vivo*.²⁴

Taken together, a set of primary proinflammatory cytokines might be induced through mechanical trauma and skin barrier disruption, initiating inflammation in atopic individuals. Furthermore, staphylococcal and mast cell products might sustain the release of these mediators and support the cycle of inflammation.

RECRUITMENT OF PATHOGENIC LEUKOCYTE SUBSETS

Next to adhesion molecules, a new family of small cytokine-like proteins, the chemokines, has attracted significant interest with regard to understanding the mechanisms of leukocyte trafficking.²⁵⁻²⁸ Chemokines are small (8-11 kd) chemotactic peptides that organize innate, as well as adaptive, immune responses.²⁸ To date, 45 human chemokine ligands have been identified, and this superfamily is thought to be among the first functional protein families completely characterized at the molecular level. On the basis of the arrangement of the amino terminal cysteine residues, chemokines can be divided into 4 subclasses: (1) CXC chemokines (termed CXC ligands

[CXCLs]), (2) CC chemokines (CCL), (3) C chemokines (XCL), and (4) CX₃C chemokine (CX₃CL).²⁷

Chemokines bind pertussis toxin–sensitive, 7-transmembrane–spanning, G protein–coupled receptors. To date, 10 CC chemokine, 7 CXC chemokine, 1 CX₃C, and 1 XCR receptors have been characterized.^{28,29} Chemokine receptor signaling involves different pathways sustaining cell survival, inducing gene expression, and, most importantly, enabling directional cell migration.²⁷

During the multistep process of leukocyte trafficking, chemokine ligand–receptor interactions mediate the firm adhesion of leukocytes to the endothelium and initiate transendothelial migration from the blood vessel into perivascular pockets.²⁵ From perivascular spaces, matrix-bound sustained chemokine gradients direct skin-infiltrating leukocyte subsets to subepidermal or intraepidermal locations.

Several homeostatic and inflammatory chemokines, including CCL1, CCL2, CCL3, CCL4, CCL5, CCL11, CCL13, CCL18, CCL20, CCL22, CCL26 and CCL27, have been shown to be associated with an AD phenotype and might support leukocyte recruitment (Table I).^{3,30-56} Moreover, serum levels of CCL11, CCL17, CCL22, CCL26, CCL27, and CX₃CL1 directly correlated with disease activity, suggesting an important role in the pathogenesis of AD (Table I).

Histopathologically, AD is characterized by an inflammatory infiltrate, mainly consisting of CD4⁺ memory T cells located in perivascular, subepidermal, and intraepidermal spaces.⁴ Next to T cells, distinct DC subsets, mast cells, and eosinophils constitute the inflammatory infiltrate.⁴

Recruitment of skin-homing memory T cells

Clinical and experimental evidence indicates that T cells play a crucial role in the immunopathogenesis of atopic skin inflammation.^{4,57,58}

The cutaneous lymphocyte–associated antigen (CLA) identifies a subset of skin-homing memory T cells.^{6,25} Specific immune responses to common skin-associated allergens, including nickel and house dust mite, are restricted to CLA⁺ T cells. CLA interacts with its vascular ligand E-selectin and mediates the rolling of distinct leukocyte subsets along the vascular endothelium.^{6,25} E-selectin is not skin specific but is expressed on inflamed endothelium of various tissues.^{6,25} Hence other skin-specific cues must regulate the tissue-specific homing capacity of CLA⁺ memory T cells.

One member of the chemokine superfamily, CCL27, is a skin-specific chemokine exclusively produced by epidermal keratinocytes.^{3,59} This novel chemokine is abundantly expressed under homeostatic conditions and inducible by proinflammatory mediators, such as TNF- α and IL-1 β .^{3,59} It shows a high binding affinity to extracellular matrix proteins and is displayed on cutaneous vascular endothelium, a phenomenon that is explained by the observation that chemokines are transported across the endothelium to participate in leukocyte arrest.^{3,59} CCL27 binds the formerly orphan G protein–coupled receptor

TABLE I. AD-associated chemokines and their receptors

Chemokine ligand	Cell of origin	Increased serum/plasma levels	Corresponding receptor(s)	Target cell(s)	References
CCL1/I-309	Keratinocytes, endothelial cells, mast cells	Yes	CCR8	T cells, LC precursors	30
CCL2/MCP-1	Keratinocytes, leukocytes	Yes	CCR2	Monocytes, DC precursors	31
CCL3/MIP-1 α	Leukocytes	Yes	CCR1, CCR5	Monocytes, T cells	31
CCL4/MIP-1 β	Leukocytes	Yes	CCR5	Monocytes, T cells	31
CCL5/RANTES	Keratinocytes, leukocytes	Yes	CCR1, CCR3, CCR5	Eosinophils, T cells, fibroblasts	31-35
CCL11/eotaxin	Keratinocytes, T cells	Yes*	CCR3	Eosinophils, T cells	34, 36-40
CCL13/MCP-4	Keratinocytes, macrophages, T cells	—	CCR2, CCR3	Monocytes, DC precursors, eosinophils, T cells	40
CCL17/TARC	Keratinocytes, endothelial cells	Yes*	CCR4	T cells	37, 38, 41-45
CCL18/PARC	Keratinocytes, DCs	Yes	Unknown	T cells	46, 47
CCL20/MIP-3 α	Keratinocytes	Yes	CCR6	T cells, monocytes, immature DC, LCs	48
CCL22/MDC	Macrophages, DCs	Yes*	CCR4	T cells	38, 49-54
CCL26/eotaxin-3	Keratinocytes, endothelial cells	Yes*	CCR3	Eosinophils, T cells	51
CCL27/CTACK	Keratinocytes	Yes*	CCR10	T cells	3, 41, 53, 55
CX ₃ CL1/fractalkine	Endothelial cells	Yes*	CX3CR1	T cells, NK cells, monocytes, DC	56

MCP, Monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TARC, thymus and activation-regulated chemokine;

PARC, pulmonary and activation-regulated chemokine; MDC, macrophage-derived chemokine; CTACK, cutaneous T cell-attracting chemokine.

*Serum or plasma levels directly correlated with disease activity.

GPR-2, which has been now defined as CCR10.⁶⁰ *In vivo*, the CCL27-CCR10 interaction regulates memory T cell recruitment to the skin, as well as allergen-specific skin inflammation.³ Neutralization of CCL27 significantly impairs inflammatory skin responses in mouse models mimicking allergic contact dermatitis and AD.³ In addition to CCR10, skin-homing CLA⁺ memory T cells abundantly express CCR4 on their cell surface.

A recent study by Reiss et al⁵ suggests that CCR4 and CCR10 ligands cooperate in the recruitment of memory T cells to sites of skin inflammation. The CCR4 ligand CCL17 is expressed by the vascular endothelium of cutaneous venules. Its expression is not specific to the skin but also detected at various noncutaneous sites.⁵ As a model, endothelial cell-derived CCL17 might cooperate with CCL27 in mediating leukocyte arrest and diapedesis. Sustained gradients of matrix-bound CCL27 can subsequently direct lymphocytes from perivascular pockets to subepidermal or intraepidermal locations (Fig 2).

A comprehensive analysis of the expression of all known chemokines in chronic inflammatory skin diseases identified CCL18 to be specifically associated with an AD phenotype but absent in other chronic inflammatory or autoimmune skin diseases, such as psoriasis or cutaneous lupus erythematosus.⁴⁶ CCL18 represented the most highly expressed ligand in AD, and the absolute amount of CCL18 mRNA in lesional atopic skin was more than 100-fold higher than that seen for CCL17.⁴⁶ In good

accordance with this finding, DNA microarray analyses also identified CCL18 as one of the genes showing the strongest association with AD skin compared with psoriatic or healthy skin specimens.¹² Interestingly, trigger factors of atopic skin inflammation, such as allergen exposure and staphylococcal superantigens, markedly induced this chemokine *in vitro* and *in vivo*, suggesting important CCL18-driven processes during the initiation and amplification of atopic skin inflammation.⁴⁶ Although its receptor is yet unknown, CCL18 binds to CLA⁺ memory T cells in peripheral blood of patients with AD *in vitro* and induces the recruitment of memory T cells into human skin *in vivo*.⁴⁷ Hence CCL18 might play a role in the organization of T-cell responses.⁴⁷ This hypothesis is supported by a recent study demonstrating that human CCL18 enhanced antigen-specific primary CD8⁺ T-cell responses when co-administered with malaria vaccines in mice.⁶¹ CCL18 is produced by dermal DCs in a close proximity to infiltrating T cells, suggesting the involvement of this chemokine in the formation of T-cell-DC clusters.⁴⁶ Taken together, CCL18, produced by antigen-presenting cells after contact with specific allergens or microbial products, might mediate the recruitment of T cells into the skin and ensure their encounter with allergen- or superantigen-loaded DCs.

Recently, Gombert et al³⁰ demonstrated that the expression of the CC chemokine CCL1 is also significantly and selectively upregulated in patients with AD in comparison with that seen in patients with psoriasis, cutaneous lupus

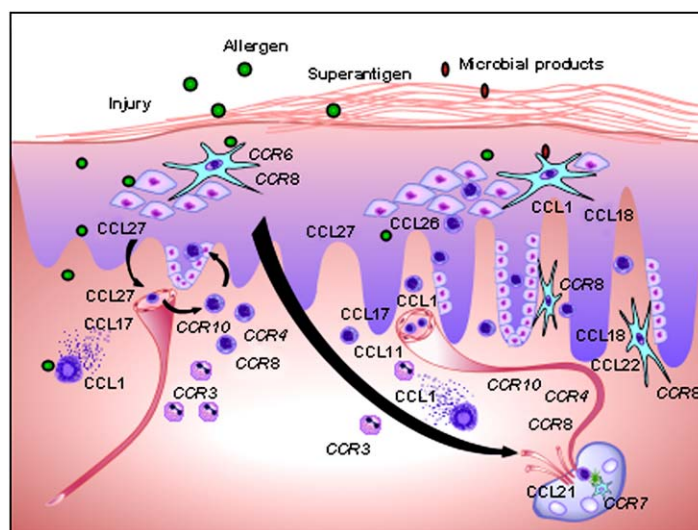


FIG 2. The complex network of chemokines in atopic skin inflammation. Findings of recent studies suggest a tightly controlled temporal-spatial expression of chemokines during atopic skin inflammation. Mechanical injury stimulates the production of primary proinflammatory cytokines (IL-1 and TNF- α), which in turn induce CCL27 and CCL17 production by keratinocytes and endothelial cells. Subsequently, CCL27 and CCL17 cooperate to recruit CCR10⁺/CCR4⁺ skin-homing CLA⁺ memory T cells into the skin. Within the skin, T cells are activated through allergens or superantigens and release effector cytokines (eg, IL-4, IL-13, or IFN- γ). This set of effector cytokines will sustain and amplify the production of chemokines within atopic skin. For example, the IL-4-inducible chemokines CCL11 and CCL26 released by keratinocytes, endothelial cells, and T cells will support the recruitment of eosinophils. Type 2 cytokines suppress antimicrobial peptide production, and colonization of atopic skin with *S aureus* might lead to bacterial superantigen-induced CCL1 and CCL18 production in DCs, keratinocytes, and endothelial cells. These chemokines induce the recruitment of skin-homing CLA⁺ memory T cells and LCs into atopic skin. Furthermore, IgE-allergen complexes trigger the release of CCL1 from mast cells. Moreover, CCR2 and CCR6 ligands sequentially contribute to the increased recruitment of DCs to sites of atopic skin inflammation. In AD lesions allergen-loaded DCs mature under the influence of proinflammatory cytokines, upregulate CCR7, and migrate through CCL21-expressing afferent lymphatics into local draining lymph nodes, where they present relevant determinants of allergen to specific T-cell subpopulations, which in turn expand, differentiate, and upregulate tissue-specific homing receptors and recirculate to extravasate at sites of allergen exposure.

erythematosus, or healthy skin. The *CCL1* gene is located in a chemokine gene cluster including 15 different ligands on chromosome 17q11.2. Many of the CC chemokines in this cluster bind the same receptors, namely CCR1, CCR2, CCR3, and CCR5.²⁸ Interestingly, although the gene encoding CCL1 is located in this chemokine gene cluster, it is the only CC chemokine from this cluster that specifically binds CCR8.²⁸ This suggests that CCL1-CCR8 interactions might mediate nonredundant functions. Recent insights into the relationship between DNA viruses and hosts showed that during the evolution of the complex virus-host interactions, viruses adapted to the immune system of their hosts and developed viral chemokine receptor antagonists to escape the host's immune defense. One such example is the epidermotropic DNA virus *Molluscum contagiosum* expressing the viral CCR8 antagonist MC148. Interestingly, *M contagiosum* infections show a markedly increased frequency in individuals with AD, suggesting that this DNA virus adapted to preferentially CCL1-CCR8-driven skin immune responses in atopic individuals. Gombert et al³⁰ also demonstrated that CCL1 serum levels of patients with AD were significantly higher than those of healthy individuals.²⁸ DCs, mast cells, and dermal endothelial cells were abundant sources of CCL1

during atopic skin inflammation, and allergen challenge, as well as *Staphylococcus aureus*-derived products, induced its production.²⁸ *In vitro*, binding and cross-linking of IgE on mast cells resulted in a significant upregulation of this inflammatory chemokine. Its specific receptor, CCR8, was expressed on a small subset of circulating T cells and was abundantly expressed on interstitial DCs, Langerhans cells (LCs) generated *in vitro*, and their monocytic precursors.²⁸ Although DCs maintained their CCR8⁺ status during maturation, brief activation of circulating T cells recruited CCR8 from intracytoplasmic stores to the cell surface.²⁸ Moreover, the inflammatory and atopy-associated chemokine CCL1 synergized with the homeostatic chemokine CXCL12, resulting in the recruitment of T (CD8⁺CD4⁺) and LC-like DCs.²⁸ CCL1 has also been described as a potent antiapoptotic factor for thymocytes, suggesting that CCL1-CCR8 interactions might provide survival signals for T cells and DCs at sites of atopic skin inflammation. These findings suggest that allergen or microbial products (*S aureus*) might trigger CCL1 and CCL18 production in atopic individuals, which in turn recruit memory T cells and in particular LCs into the skin and lead to their accumulation in subepidermal and intraepidermal locations. Because activated DCs secrete large amounts of CCL1,

CCL1-CCR8–driven recruitment pathways might facilitate DC–T-cell interactions at sites of atopic skin inflammation. Moreover, CCR8 signaling might enhance cell survival, providing a potential mechanism that sustains atopic inflammation and prevents activation-induced apoptosis of skin-infiltrating leukocytes. Thus CCL1 and CCL18 might provide pathways linking adaptive and innate immune functions, leading to the accumulation of relevant leukocyte subsets at sites of atopic skin inflammation and supporting the initiation and amplification of AD.

DC trafficking

DCs serve as sentinels of the immune system and represent a heterogeneous family of cells. Their precursors migrate from postcapillary venules into peripheral tissues, differentiate, and capture, while in an immature state, antigens or allergens. After stimulation with proinflammatory mediators, DCs mature and leave peripheral sites, enter afferent lymphatics, and migrate to local draining lymph nodes, where they prime naive T cells and initiate immune responses.^{62,63} During their activation and differentiation, T cells critically depend on the stimulation of antigen-presenting cells to differentiate into effector cells. In AD an increased number of DCs has been observed both within the epidermis and the dermis.^{4,64} Next to LCs, inflammatory dendritic epidermal cells constitute the DC population observed within the epidermis.⁴ Tissue macrophages and interstitial-dermal DCs represent professional antigen-presenting cells within the dermal compartment of patients with AD.⁴

The important role of DC migration during the development of immune responses led to extensive analyses of the chemokine receptor repertoire and chemokine responsiveness of DC populations during their development and maturation. Overall, immature DCs respond to many CC and CXC chemokines, including CCL3, CCL4, CCL13, CCL5, CCL20, CCL25, and CXCL12, but each immature DC subpopulation shows a unique spectrum of chemokine responsiveness.⁶²

Immature DCs can roughly be divided into 4 distinct groups of related cells: epithelial DCs (LCs), interstitial-dermal DCs, monocyte-derived DCs, and CD11c[−] DC precursors. In contrast to the lesional skin of patients with psoriasis or lupus erythematosus, the skin of patients with AD does not show an accumulation of CD11c[−]/plasmacytoid DCs. *In vitro*, Langerhans-type or interstitial-dermal-type DCs can be generated from either monocytes or CD34⁺ hematopoietic progenitor cells. Peripheral blood DCs and monocytes display many chemokine receptors, including CCR1, CCR2, CCR5, CXCR4, and CX₃CR1, on their cell surface but primarily respond to CCR2 ligands.⁶² In contrast, *ex vivo*–derived LCs or *in vitro*–generated LC-type DCs demonstrate a restricted chemokine receptor repertoire, with CCR6 being the only abundantly expressed receptor on this DC subset.^{62,64} Because no CCR6⁺ populations are detected among putative DC precursors within the peripheral blood, a model of sequential chemokine responsiveness has been suggested.⁶⁵ Blood DCs or DC precursors are recruited into peripheral tissues (ie, skin)

through CCR2 ligands expressed in the context of endothelial cells and blood vessels. Within perivascular spaces, DC precursors undergo differentiation processes induced by the tissue microenvironment (ie, TGF- β) and gain CCR6 expression.⁶⁵ Subsequently, CCL20 production by keratinocytes at sites of injury or allergen exposure might direct LC precursors into the epidermis and lead to their accumulation in AD.⁶⁵ Indeed, Nakayama et al⁴⁸ showed that CCL20 is weakly expressed in healthy skin but markedly produced by keratinocytes in the lesional skin of patients with AD and associated CCL20-CCR6–driven pathways with the accumulation of immature DCs and memory T cells within atopic skin.

In summary, the following ligands have been shown to be induced and are likely candidates to recruit DC precursors from the circulation to sites of atopic skin inflammation: CCL1, CCL2, CCL5, CCL13, CCL20, and CX₃CL1.

Once in peripheral tissues, immature DCs function as sentinels of the immune system. On stimulation with inflammatory mediators, such as IL-1, TNF- α , CD40 ligand (CD40L), virus, or bacterial products, DCs mature and undergo phenotypic changes. Uniformly, mature DC subpopulations lose their ability to capture antigen and chemotactic responsiveness toward inflammatory chemokines but upregulate the chemokine receptor CCR7.⁶² In turn, CCR7 ligands (eg, CCL19 and CCL21) are abundantly expressed in afferent lymphatics and with secondary lymphoid tissues directing DC migration to local draining lymph nodes.⁶²

Taken together, DCs and their precursors undergo tightly controlled migration processes. A number of chemokines expressed in atopic skin inflammation might explain the accumulation of dermal, as well as epidermal, DC populations in AD. Their temporal-spatial expression pattern might decide the composition and anatomic distribution of DCs in the different phases of atopic skin inflammation.

Recruitment of eosinophils

Increased numbers of circulating eosinophils are frequently observed in patients with AD.⁴ A survey on the chemokine receptor repertoire of human peripheral blood eosinophils draws a confusing picture of receptors, including CCR1, CCR2, CCR3, CCR4, CCR5, CCR9, CXCR3, and CXCR4, which are reported by some investigators, but other studies do not confirm their expression. However, there is increasing evidence that CCR2, CCR3, and CXCR4 are expressed on the cell surface of human eosinophils and induce significant chemotactic responses. Among all chemokine receptors associated with eosinophils, CCR3 represents the most extensively studied member of this protein family. CCR3 represents a highly promiscuous receptor binding at least 9 different human chemokine ligands. Among those, CCL5, CCL11, CCL13, and CCL26 have been reported in AD.

A recent study by Yawalkar et al³⁶ demonstrated the expression of CCL11 and CCR3 in patients with AD. Immunoreactivity and transcripts of CCL11 and CCR3 were significantly increased in lesional skin from patients with AD but not in nonatopic control subjects. CCL11 and

CCR3 immunoreactivity was predominantly seen in mononuclear cells of the dermis.³⁶ Analysis of serial sections suggested that CD3⁺ lymphocytes were major producers of these proteins. In addition, mononuclear cells, fibroblasts, and eosinophils were identified as producers of CCL11 in lesional atopic skin.³⁶ Thus the authors proposed a positive feedback loop that might preferentially amplify the chemotactic response of T lymphocytes and eosinophils. Furthermore, CCL5 protein could be detected at high levels in skin scales of patients with AD, and dermal fibroblasts were identified as a major source of this chemokine. Moreover, Gluck and Rogala³² demonstrated that CCL5 serum levels were significantly increased in patients with AD compared with in healthy nonatopic control subjects. However, serum levels did not correlate with clinical scores of patients. In contrast, serum levels of CCL11, CCL13, and CCL26 were significantly upregulated in patients with AD and directly correlated with disease activity (Table I).

Experiments in CCR3-deficient mice recently demonstrated that this chemokine receptor is essential for skin eosinophilia and airway hyperresponsiveness in a murine model of AD.⁶⁶ Although eosinophils and the eosinophil product major basic protein were absent from the skin of sham and ovalbumin-sensitized CCR3^{-/-} mice, mast cell numbers and the expression of IL-4 mRNA were normal within the skin, suggesting that CCR3 is not essential for the infiltration of mast cells and T_H2 cells into the skin.⁶⁶ Furthermore, CCR3^{-/-} mice produced normal levels of ovalbumin-specific IgE, and their splenocytes secreted normal amounts of IL-4 and IL-5 after *in vitro* stimulation with ovalbumin, indicating effective generation of systemic T_H2 responses.⁶⁶ Hence findings of this study suggest that CCR3 ligands are critical for eosinophil recruitment during atopic skin inflammation but might not play a dominant role in T-cell differentiation and recruitment.

In summary, these findings suggest that CCR3-driven pathways are essential for the recruitment of eosinophils to sites of atopic skin inflammation. In the lesional skin of patients with AD, CCR3 ligands, including CCL5, CCL11, CCL13, and CCL26, might be candidates to mediate the influx of eosinophils into the skin.

Taken together, within the complex puzzle of mediators regulating leukocyte trafficking under homeostatic and inflammatory conditions, a concept emerges suggesting that an orchestrated process of temporal-spatial expression of adhesion molecules and chemokines is responsible for the recruitment and accumulation of leukocytes at peripheral sites, such as atopic skin (Fig 2). Although accumulating studies have provided significant insight on the association of chemokines with an AD phenotype, comparably little information exists on their functional role *in vivo*.

T-CELL DIFFERENTIATION AND ACTIVATION

For nearly a decade, the concept of type 1 and type 2 (T_H1 and T_H2) T-cell responses has been applied to atopic diseases. Type 1 responses are initiated by IL-12 and

characterized by T lymphocytes predominantly producing the effector cytokine IFN- γ . Conversely, type 2 T-cell differentiation is driven by IL-10 and prostaglandin E₂ and characterized by the production of IL-4, IL-5, and IL-13. Overall, atopic diseases have been associated with a T_H2 phenotype, showing dominance of IL-4, IL-5, and IL-13 secretion; blood eosinophilia; and increased serum IgE levels. In addition to important proinflammatory functions, IL-4 mediates the IgE isotype switch in B cells. Recent observations in acute and chronic lesions of patients with AD, together with kinetic studies using the atopy patch test, indicate that although the initiation of atopic skin lesions is driven by IL-4-producing T_H2 cells, chronic lesions show either the coexistence of both IL-4-producing type 2 and IFN- γ -producing T_H1 cells or T_H1 dominance.⁶⁷⁻⁶⁹ Furthermore, the presence of IFN- γ -producing T cells correlates with the chronification and severity of AD skin lesions.⁶⁷⁻⁶⁹

For a long time, the role of keratinocytes during the initiation of allergic responses has been an enigma. Soumelis et al¹¹ demonstrated that epithelial cells trigger DC-mediated allergic inflammation by producing the novel IL-7-like hematopoietic cytokine TSLP (Fig 3). TSLP is abundantly produced by keratinocytes in the lesional skin of patients with AD. TSLP-activated human CD11c⁺ DCs prime T cells to produce IL-4, IL-5, and IL-13 but no IL-10 or IFN- γ .¹¹ Furthermore, stimulation of human CD11c⁺ DCs with this novel hematopoietic cytokine results in the release of CCL17 and CCL22, 2 atopy-associated chemokines that preferentially attract CCR4⁺ T_H2 cells.¹¹ Next to CD4⁺ T-cell responses, TSLP-activated human CD11c⁺ DCs potently activated naive CD8⁺ T cells to expand and differentiate into IL-5- and IL-13-producing effector cells.⁷ Additional CD40L triggering of TSLP-activated DCs induced CD8⁺ T cells with potent cytolytic activity, producing large amounts of IFN- γ while retaining their capacity to produce IL-5 and IL-13.⁷ Hence TSLP might represent an initial trigger of atopic T-cell responses, and this suggests that in concert with CD40L-expressing cells, TSLP might amplify and sustain proallergic responses and cause tissue damage by promoting the generation of IFN- γ -producing cytotoxic effectors.

Together, these findings suggest that the perception of epithelial cells as pure lining cells might change and include effector functions determining the direction and type of immune responses.

Recently, 2 independent *in vivo* studies underscored the important role of TSLP in the development of AD-like skin inflammation.^{8,10} Inducible skin-specific overexpression of TSLP resulted in an AD-like phenotypes with the development of dermatitis lesions on the snout, postauricular region, nape of the neck, and upper back.¹⁰ Generalized xerosis cutis and conjunctivitis were also observed.¹⁰ Histopathologic examinations of eczematous lesions showed acanthosis, spongiosis, hyperkeratosis, and a dermal mononuclear infiltrate containing markedly increased numbers of lymphocytes, macrophages, eosinophils, and mast cells.¹⁰ In addition to the skin phenotype, mice showed increased IgG1 and IgE levels within the serum.¹⁰

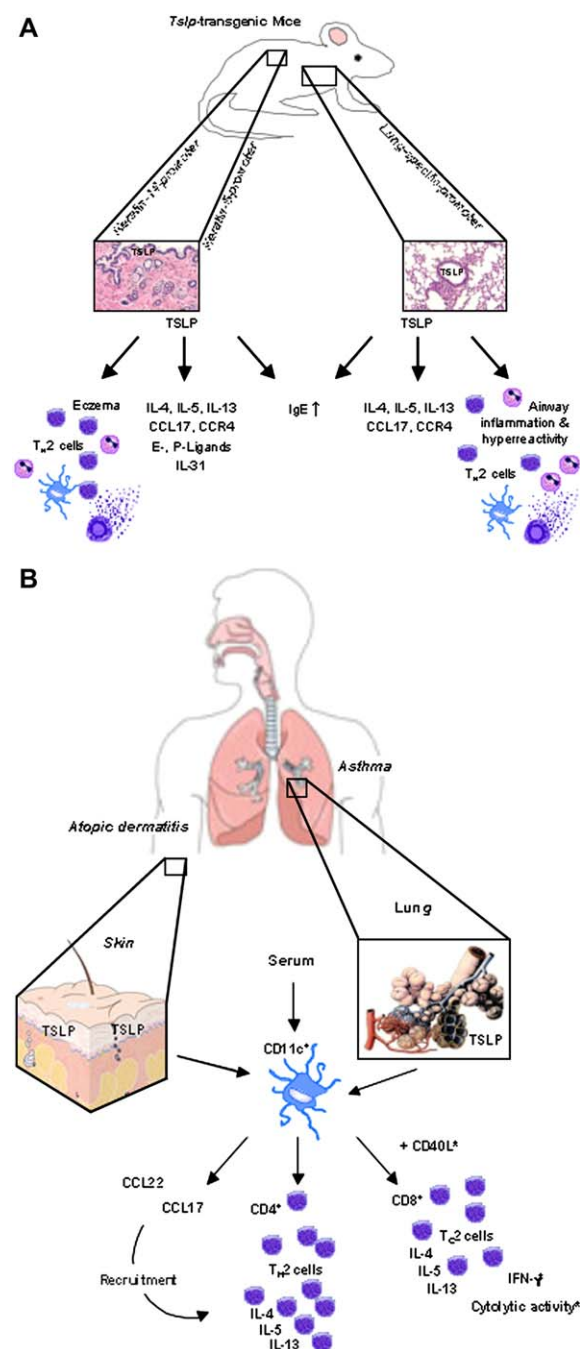


FIG 3. Epithelial cells trigger DC-mediated allergic inflammation by producing TSLP. **A**, In patients with AD, TSLP is abundantly produced by keratinocytes *in vivo*. *In vitro* TSLP-activated human CD11c⁺ DCs prime CD4⁺, as well as CD8⁺, naive T cells to differentiate and produce IL-4, IL-5, and IL-13. Additional CD40L triggering of TSLP-activated DCs induces CD8⁺ T cells with potent cytolytic activity, producing large amounts of IFN- γ while retaining their capacity to produce IL-5 and IL-13. Furthermore, stimulation of human CD11c⁺ DCs with this novel hematopoietic cytokine results in the release of CCL17 and CCL22, 2 atopy-associated chemokines that preferentially attract CCR4⁺ T_H2 cells. In asthmatic subjects TSLP is expressed within the bronchial epithelium and correlates with the expression of the T_H2-attracting chemokines (CCL17 and CCL22) and disease severity. **B**, In mice skin-specific overexpression (keratin-5 or keratin-14 promoters) of Tslp results in an

Furthermore, a striking increase of the skin-homing receptors P- and E-selectin ligands on T_H2 cells was observed in K5-TSLP mice.¹⁰ In skin lesions the expression of the atopy-associated chemokine CCL17 and its receptor CCR4 were substantially increased.¹⁰ Interestingly, crossing of K5-TSLP mice with T-cell receptor $\beta^{-/-}$ mice showed that the eczematous skin phenotype was independent of functional T cells showing epidermal thickening and a dermal inflammatory infiltrate, mainly consisting of mast cells and eosinophils.¹⁰ K5-TSLP/T-cell receptor $\beta^{-/-}$ mice demonstrated little or no serum IgE, demonstrating that T cells are necessary for the production of this isotype and that B cells do not contribute greatly to the overall phenotype seen in these mice.¹⁰

Similar to Yoo et al,¹⁰ the findings of Li et al⁸ further supported the important role of TSLP in the development of atopic skin inflammation. Overexpression of TSLP under the regulation of the keratin-14 promoter (K14-TSLP mice) resulted in the development of pruritic eczematous lesions on the ears, face, and neck region of mice.⁸ Skin lesions showed numerous CD4⁺ T cells, CD11c⁺ DCs, and eosinophils.⁸ Lesions showed a molecular signature with increased levels of IL-4, IL-5, IL-13, IL-10, and IL-31 transcripts.⁸ Moreover, serum levels of IgE were increased.⁸

Taken together, transgenic mice overexpressing TSLP within the skin demonstrated that TSLP can initiate a cascade of inflammation that leads to the development of AD-like skin lesions. Interestingly, TSLP is also increased in asthmatic airway epithelial cells, and TSLP expression correlates with disease severity in asthmatic patients, whereas lung-specific overexpression of TSLP has been shown to represent a key initiator of allergic airway inflammation in mice (Fig 3).^{9,70,71}

INNATE IMMUNE RESPONSES AND THE AMPLIFICATION OF ATOPIC SKIN INFLAMMATION

Evolutionary pressure developed and optimized an innate immune system to mount rapid responses against infectious agents through Toll-like receptors acting as pattern-recognition systems, antimicrobial peptides, or the production of proinflammatory cytokines.

Antimicrobial peptides known as cathelicidins (LL-37) and β -defensins represent another integral component of

AD-like phenotype, with the development of eczematous lesions showing acanthosis, spongiosis, hyperkeratosis and a dermal mononuclear infiltrate containing markedly increased numbers of lymphocytes, macrophages, eosinophils, and mast cells. In skin lesions the expression of the atopy-associated cytokines IL-4, IL-5, IL-13, IL-10, and IL-31, as well as the chemokine CCL17 and its receptor CCR4, is substantially increased. Furthermore, a striking increase of the skin-homing receptors P- and E-selectin ligands on T_H2 cells was observed. In addition to the skin phenotype, mice show increased serum IgE levels. In parallel, lung-specific expression of a Tslp transgene under control of the surfactant protein C promoter induced airway inflammation and hyperactivity characterized by leukocyte infiltration, goblet cell hyperplasia, subepithelial fibrosis, T_H2 cytokines, and increased IgE levels.

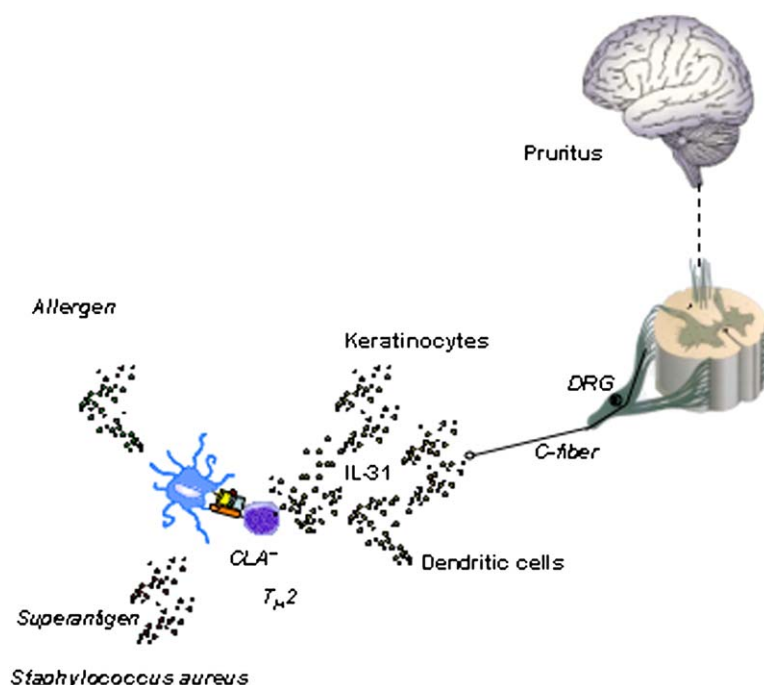


FIG 4. IL-31 linking T cells and pruritus in atopic skin inflammation. T_H2 lymphocytes preferentially express IL-31, a novel cytokine inducing pruritus and chronic dermatitis in mice. In human subjects staphylococcal superantigens markedly enhance IL-31 production. IL-31 binds a receptor heterodimer composed of IL-31RA and the oncostatin M receptor (OSMR). Potential IL-31 receptor-bearing target cells include keratinocytes, DCs, and peripheral sensory neurons, which in turn might mediate pruritic signals through direct or indirect pathways to the central nervous system.

the innate immune system of human skin.^{13,72,73} The combination of cathelicidins and human β -defensin 2 shows synergistic antimicrobial activity against *S aureus*.¹³ Recently, a new antimicrobial peptide, human β -defensin 3, has been identified, which has potent killing activity against *S aureus* by itself and can kill a broad spectrum of microbes.^{72,74} In healthy skin these peptides are expressed at low levels, but they accumulate in skin affected by inflammatory diseases, such as psoriasis. Two recent studies show that the presence of antimicrobial peptides is significantly decreased in acute and chronic lesions of patients with AD.^{13,75} *In vitro* IL-4 and IL-13 are potent inhibitors of TNF- α - and IFN- γ -induced human β -defensin 2 and β -defensin 3 expression.^{13,75} Hence the deficiency of antimicrobial peptide production might account, at least in part, for the susceptibility of patients with AD to skin infections; promote bacterial colonization (eg, *S aureus*); and, through exposure to microbial products (eg, superantigens, peptidoglycan, and lipoteichoic acid), significantly contribute to the amplification of atopic skin inflammation.

THE COMPLEX NETWORK OF MEDIATORS REGULATING PRURITUS IN ATOPIC INDIVIDUALS

Pruritus is an essential feature of AD, represents a bothersome symptom, and has a high effect on quality of

life. In general, pruritus can be divided into 4 clinical categories: (1) pruritoceptive itch originating in the skin caused by inflammation, dryness, or skin damage; (2) neuropathic itch arising from diseases affecting the afferent neuronal pathways; (3) neurogenic itch developing in the central nervous system through mediation of μ -opioid receptors; and (4) psychogenic itch caused by various psychologic and psychiatric conditions, including emotional stress.⁷⁶ In patients with AD, pruritoceptive, neurogenic, and psychogenic itch appears to be relevant.² Neuropathophysiologically, excitation of neuropeptide-containing free nerve endings of unmyelinated nociceptor fibers (C fibers) transmits pruritus.^{77,78} Various studies have shown that the number of sensory and neuropeptide-containing nerve fibers is increased in lesional AD skin.^{2,79} It is also well known that histamine and acetylcholine provoke itch through direct binding to "itch receptors."²

In addition to histamine, several other mediators, such as neuropeptides, proteases, kinins, and cytokines, can induce pruritus.^{2,15} Mast cells store large amounts of proteases, including tryptase and chymase, in their cytoplasm. Recently, a new mechanism was demonstrated in which activated dermal mast cells localized in the proximity of afferent unmyelinated C fibers directly induce pruritoceptive itch.¹⁶ Through the activation of the G protein-coupled receptor proteinase-activated receptor 2, tryptase transmits itch perception and in parallel mediates neuropeptide release, which in turn might activate mast cells through neurokinin receptors.¹⁶ Importantly, although

histamine concentrations were not increased in patients with AD, the concentration for tryptase and the density of nerve fibers was significantly enhanced in the lesional skin of patients with AD.¹⁶ Thus the tryptase-induced and proteinase-activated receptor 2-mediated pruritus might, at least in part, explain why nonsedative antihistamines show only limited antipruritic effects in some patients with AD and might direct us to future therapeutic strategies for pruritus in patients with AD.

Until recently, the contribution of T cells, which predominate in the inflammatory infiltrate seen in AD lesions, to the development of pruritus remained an enigma. A recent study by Dillon et al¹⁴ demonstrated that transgenic overexpression of the novel 4-helix bundle cytokine IL-31 in lymphocytes induces severe pruritus and dermatitis in mice. IL-31 is preferentially expressed by T_H2 cells and signals through a heterodimeric receptor composed of IL-31 receptor A (IL-31RA) and oncostatin M receptor (OSMR).^{14,80} Subsequently, studies by Bilsborough et al⁸¹ and Sonkoly et al⁸² extended these observations and showed that human IL-31 is significantly upregulated in “pruritic” (AD and prurigo nodularis) but not in “nonpruritic” (psoriasis) forms of chronic skin inflammation. Within the inflammatory infiltrate of patients with AD, inflammatory cells of the lymphocytic lineage, with the majority staining for CLA and CD3, overexpressed IL-31 mRNA.⁸¹ *In vivo* exposure to staphylococcal superantigen rapidly induced IL-31 expression in atopic individuals. *In vitro* IL-31 was induced by staphylococcal superantigens but not by T_H1- or T_H2-type cytokines or viruses in leukocytes⁸² (Fig 4). In atopic individuals activated leukocytes expressed higher levels of IL-31 transcripts compared with those seen in healthy nonatopic subjects.⁸² These observations were in line with findings of Bilsborough et al,⁸¹ showing that circulating CLA⁺ memory T cells of patients with AD were capable of producing increased levels of IL-31 protein when compared with T cells of psoriatic donors.

Expression analyses suggest that the heterodimeric IL-31 receptor (IL-31RA/OSMR) is expressed on monocytes, DCs, and epithelial cells, including keratinocytes.^{14,81,82} In cultured human keratinocytes IL-31 activates signal transducer and activator of transcription 3 and induces, among other genes, the expression of a distinct set of atopy-associated chemokines, such as CCL1, CCL17, and CCL22.¹⁴ More recently, Sonkoly et al⁸² reported that OSMR and IL-31RA transcripts are also abundantly expressed in dorsal root ganglia, the anatomic location in which the cell bodies of the primary sensory neurons reside.⁸³

Taken together, these findings provide the missing link between increased *S aureus* colonization, subsequent T-cell recruitment-activation, and pruritus induction to close the amplification cycle in atopic skin inflammation.

CONCLUSIONS AND PERSPECTIVE

Recent insights into the complex network of cytokines and chemokines in AD might provide promising

candidates for the diagnosis and molecular classification of subtypes of this disease. Furthermore, polymorphisms in cytokine or chemokine genes might represent susceptibility factors for the development of AD. Indeed, recent studies suggest a link; however, further studies with large and well-characterized patient and control collectives are necessary to confirm and validate these, as well as upcoming, findings.

Although findings of recent studies substantially enhanced our understanding of the immunopathogenesis of AD, significant therapeutic challenges remain. In particular, the long-term management of patients with severe, chronically relapsing inflammatory skin diseases still represents a significant unmet medical need. Novel chemokine antagonist-based strategies to interfere with skin inflammation are likely to be preventive rather than therapeutic. Chemokine antagonists hold promise to provide excellent tools to impair the recruitment of pathogenic leukocyte subsets to the skin or other peripheral sites. Once leukocytes have entered their target organ and undergone activation processes, chemokine antagonists are likely to be less effective.

Together with the availability of potent drugs to treat atopic skin inflammation, such as glucocorticosteroids, topical immunomodulators (tacrolimus and pimecrolimus), or cyclosporine, chemokine-receptor antagonists might represent promising candidates to reduce the frequency of acute flares, prolong the lesion-free interval, and provide optimized long-term management of patients with chronically relapsing inflammatory skin diseases, such as AD.

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