Modifications of the Innate Immune System in Atopic Dermatitis

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Key Words

Allergy · Antimicrobial peptides · Pathogen-associated molecular patterns · Toll-like receptor

Abstract

Atopic dermatitis (AD) is a frequent chronic inflammatory skin disease which is often complicated by recurrent microbial superinfections. Genetically based modifications which might have an impact on the innate immune system, such as impairment of the skin barrier, modifications of pattern recognition receptors, deficiency of antimicrobial peptides, antiviral natural killer cells and plasmacytoid dendritic cells, facilitate the entry of allergens and infectious microbes into the skin, where they encounter immunocompetent cells. The microclimate in the skin of AD patients further potentiates dysfunctions of the innate immune system, leading to a vicious circle promoting the disease. This article provides an overview of modifications of the innate immune system in AD.

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Introduction

Atopic dermatitis (AD) is a multifactorial chronic inflammatory skin disease characterized by pruritic, typical distributed eczematous skin lesions, disturbed skin barrier and modified innate and adaptive immunity with

a proneness to IgE-mediated sensitization, infections and hyperreactivity to environmental influences with a strong genetic predisposition. Current studies revive the question whether the disturbed epidermal barrier in AD leads to modified innate and adaptive immune responses (inside-outside), or the other way round (outside-inside paradigm).

Innate immunity consists of cellular and biochemical defense mechanisms that provide a rapid response to invasion of microbes after their recognition by pattern recognition receptors (PRR). The principal components of innate immunity are physical and chemical barriers – such as epithelia and antimicrobial substances produced at epithelial surfaces, circulating effector cells [neutrophils, macrophages, natural killer (NK) cells], circulating effector proteins such as mannose-binding lectin, and cytokines such as tumor necrosis factor (TNF), interleukins (IL)-1, -12, -18, -23, -15 and -10, chemokines, interferons (IFN)- α , - β and - γ , and transforming growth factor- β – that regulate and coordinate many of the activities of the cells of innate immunity. Defense against microbes is mediated by the early reactions of innate immunity and the later responses from adaptive immunity. Alterations in both innate and adaptive immunity have been described in AD, leading to frequent bacterial, viral and mycotic superinfections which trigger and aggravate the course of the disease. This article aims to provide an overview of modifications of the innate immune system in AD discovered in recent times.

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Fig. 1. Diagram of the epidermis. Adapted from Candi et al. [2]. BPAG = Bullous pemphigoid antigen; CE = cornified envelope; SC = stratum corneum; SPR = small proline-rich protein; TG = transglutaminase.

Genetically Based Skin Barrier Dysfunction in AD

A modified skin barrier with increased transepidermal water loss and reduced hydration of the skin is a characteristic feature of AD patients, whereupon the extent of barrier dysfunction correlates with AD severity [1]. The epidermis functions both as a physical barrier and as an active immunological organ. During differentiation, keratinocytes (KC) move from a proliferative cell type in the basal cell layer of the epidermis through the granular layer, where the cornified envelope (CE) is formed, to an association of flattened, dead cell remnants (corneocytes) in the uppermost layer of the skin, the stratum corneum (SC). The CE is an insoluble protein structure that is cross-linked by transglutaminases to replace the plasma

membrane in corneocytes, where it functions as a scaffold for lipid attachment. This structure prevents epidermal water loss and also impedes the entry of allergens, irritants and infectious organisms. A matrix of lipids such as ceramides, cholesterol, fatty acids and cholesterol esters compasses the corneocytes and builds the critical barrier to transepidermal water loss. Corneodesmosomes function as adhesion molecules between the corneocytes (fig. 1) [2]. AD patients exhibit a reduced expression of ceramides and CE proteins such as involucrin, loricrin, filaggrin (filament-aggregating protein, FLG) and keratin K5 and K16 in both lesional and nonlesional skin [3].

In the last years, FLG has gained outstanding interest due to the strong association of AD with polymorphisms in the *filaggrin* gene (*FLG*; chr1q21, epidermal differen-

tiation complex), which have been replicated in multiple case-control and family studies, leading to a paradigm shift from a primary immunological disease towards a dysfunction of the epidermal barrier [4].

FLG consolidates the keratin filaments into dense bundles, represents an integral part of the epidermis and is crucial for the development of the CE to engineer and maintain the barrier function of the uppermost layer of the skin. Moreover, FLG degradation products such as hygroscopic amino acids serve as a natural moisturizing factor, and acidic metabolites of FLG can influence the pH of the skin and increase serine protease (SP) activity. The *FLG* mutations 228del4 and R501X are the main *FLG* variants in Europeans, with a combined allele frequency of about 6%. Moreover, 18 infrequent variants have been found in populations with European ancestry, and an additional 17 mutations in Asian populations. All variants lead to nonsense mutations and impede or rather decrease the production of free epidermal FLG [4]. *FLG* null mutations have been described in 16–23% of all AD patients in central European studies, and in 22–45% in studies from the UK. The strongest association with *FLG* mutations have been observed in AD patients with high total serum IgE, concomitant sensitizations and an early onset of AD with a chronic, persistent course until adulthood. Combined analysis shows an increased risk of *FLG* haploinsufficiency for both eczema as well as for the combined phenotype of AD with asthma, but not for asthma without eczema [4]. The effect of FLG on the risk of eczema is considerably higher than the effect of other candidate genes on atopic diseases, and it is one of the strongest effects reported and replicated in the genetics of complex diseases [4].

Besides mutations in the *FLG* and several genes for atopy and inflammation, AD has also been associated with mutations in other genes for proteins contributing to the skin barrier, such as the SP SC chymotryptic enzyme, kallikrein-related peptidase 7 (*KLK7*) and the SP inhibitor LEKTI (*SPINK5*), which contribute to the balance of proteases and antiproteases in the skin, and *CLDN1*, encoding claudin 1. The tight junction protein claudin 1 controls the permeability of epithelial cells to water and other soluble components and immune cells and is decreased in patients with AD [5].

SP are involved in the proteolytic degradation of corneodesmosomes and, together with lipid-processing enzymes such as sphingomyelinases, in the desquamation of the corneocytes from the SC surface. Sustained SP activity by a slight, prolonged alkalinization in pH from 5.0 to 5.5 in AD patients is suspected to induce abnormalities

in both SC integrity, cohesion and permeability barrier homeostasis. SP mediate proinflammatory effects via the proteinase-activated receptor 2 (PAR2), which is highly expressed on KC and dermal endothelial cells. These cells react to PAR2 signals with hyperproliferation and an increased expression of proinflammatory cytokines and chemokines. Moreover, activation of PAR2 on human skin mast cells induces the release of histamine. Acute barrier disruption increases SP activity and activates PAR2. Trypsin and trypsin-related enzymes act as endogenous PAR2 activators, tryptic enzymes from *Staphylococcus aureus* and house dust mite (HDM) as exogenous PAR2 activators. Besides the induction of an allergen-specific immune reaction, HDM might thus elicit an additional nonimmunologic inflammatory reaction in both sensitized and nonsensitized individuals.

The partly genetically based skin barrier dysfunction permits an invasion of the skin by antigens, bacteria and viruses, where they encounter immunocompetent epidermal and dermal cells and thus influence the course of the disease. Fitting into this, *FLG*-deficient mice display an increased uptake of allergens through the skin, leading to an IgE-mediated sensitization and development of an atopic skin inflammation with an overexpression of IL-17, -6, -23 and -4 as well as IFN- γ [6]. However, 40% of patients with *FLG* null alleles do not suffer from AD, and null mutations causing the *FLG* deficiency could only be demonstrated in about 1/3 of the patients. Interestingly, Th2 cytokines have been shown to downregulate the expression of *FLG* [7], other CE proteins such as loricrin and involucrin [8], and also ceramide synthesis [9]. Thus, a part of the AD patients seems to acquire the skin barrier dysfunction by atopic inflammatory response, or the genetic predisposition to a dysregulated skin barrier is amplified by the inflammatory microenvironment in AD [7].

Mutations in Genes for PRR

Via special PRR, the innate immune system recognizes highly conserved pathogen-associated molecular patterns (PAMP), which are shared by groups of related microbes and which do not occur in humans. This leads to the rapid activation of cellular and biochemical defense mechanisms of the innate immune system and subsequent reactions of the adaptive immune system. Several PRR such as Toll-like receptors (TLR) or intracellular nucleotide-binding oligomerization domains (NOD)/caspase recruitment domains (CARD) distinguish between different PAMP. TLR are expressed by various cells of the

innate immune system such as macrophages, dendritic cells (DC), neutrophils, mucosal epithelial and endothelial cells. Recognition of their ligands induces a conserved host recognition program via nuclear factor- κ B, leading to the expression of proinflammatory cytokines (TNF- α , IL-1, IL-12), endothelial adhesion molecules (E-selectin), costimulatory molecules and antimicrobial mechanisms [antimicrobial peptides (AMP), inducible nitric oxide synthase]. Activation of DC by TLR ligands is crucial for their maturation and ability to initiate adaptive immune responses. Weak TLR2 and TLR4 signals in the context of allergen exposure in the skin and lung, respectively, have been shown to promote a Th2-based immune response [5]. TLR2 recognizes fungal zymosan, lipopolysaccharides and other PAMP such as components of the staphylococcal cell wall [lipoteichoic acid (LTA), peptidoglycan (PGN)], and is thus essential for the response to several bacteria, mycobacteria, protozoa and fungi. Associations of the single nucleotide polymorphisms (SNPs) R753Q [10] and A-6934T [11] in the *TLR2* gene with severe forms of AD have been described [10], but the association of R753Q could not be replicated in another study [12]. Furthermore, no association of AD could be shown with SNPs in the *TLR3* [13], *TLR4* [11, 12] or *TLR6* gene. There were no significant differences in mRNA expression of TLR 1, 2, 3, 5 and 6 between skin biopsies of patients with AD and psoriasis [14]. However, lower TLR2 expression was observed on macrophages of patients with AD, and macrophages of patients with AD produced less proinflammatory cytokines (IL-6, -8, - β) after stimulation with PGN and LTA [15]. Heterozygous carriers of *TLR2* R753Q with AD displayed a reduced surface expression of TLR2 on CD4+ T cells [16], a modified expression of CD36 upon stimulation and an increased production of IL-6 and IL-12 by monocytes after TLR2 stimulation compared to wild-type AD patients and healthy controls [15]. Furthermore, an increased secretion of IL-6 in homozygous carriers of *TLR2* -16934A with AD has been observed [11], indicating a modified inflammatory response dependent on this *TLR2* polymorphism [15]. Furthermore, AD has been associated with a polymorphism (C-1237T) resulting in higher promoter activity in the gene encoding TLR9, which is crucial for the recognition of unmethylated CpG DNA sequences of bacteria, protozoa and intracellular viral antigens [17].

There was no evidence for an association of AD with *IRAK-M* SNPs encoding an IL-1 receptor-associated kinase M which negatively regulates TLR signaling [18]. Intracellular PAMP, particularly PGN, are recognized by the NOD family of proteins. NOD1- and NOD2-express-

ing KC produced IL-6 after stimulation with PGN, and human β -defensin (hBD)2 after stimulation with the NOD2-specific ligand muramyl dipeptide. SNPs of *CARD4* (encoding NOD1) [19], *NALP12* (another member of the NOD-leucine rich repeat-containing protein family) [20] and *CARD15/NOD2* [20, 21] have been observed to be associated with AD, whereas variants of *CARD15/NOD2* also modified the risk of developing asthma or allergic rhinoconjunctivitis [21, 22].

The circulating PRR mannose-binding lectin (MBL) recognizes mannose-rich glycans of various pathogens including *S. aureus*, leading to opsonization and complement activation. In children with AD, an association of SNPs in the *MBL* gene with AD, related to low or deficient levels of MBL, has been observed compared to healthy controls [23]. Thus, genetically determined low or deficient MBL serum levels may contribute to the predisposition to AD, but not to disease severity [23]. However, a previous Japanese study could not show any association of *MBL* SNPs with AD [24].

Deficiency of Antimicrobial Peptides (AMP) Contributes to Susceptibility to Skin Infections in AD

Modified mechanisms of the innate immune system with a deficiency of the AMP human cathelicidin LL-37, hBD2 and hBD3 contribute to the proneness of AD patients to skin infections. The cationic AMP interact with anionic components of bacteria, fungi and viruses, leading to a destruction of the microbial membrane and cell lysis. Moreover, AMP induce the production of several cytokines and chemokines which contribute to the recruitment of neutrophils, monocytes, mast cells and T cells in the skin [25]. LL-37 and hBD2 show synergistic effects in the elimination of *S. aureus* [26]. Low amounts of AMP, apart from the constitutively expressed hBD1, are expressed in healthy skin, but the synthesis of hBD2 and 3 and of LL-37 by KC increases as a reaction to *S. aureus* and proinflammatory cytokines [14, 26]. Thus, increased amounts of AMP have been found in the skin of patients with chronic inflammatory skin diseases such as psoriasis and contact dermatitis. In contrast, both lesional and nonlesional skin of AD patients [14] features a decreased expression of hBD2 and 3 and of LL-37 compared to the skin of psoriasis patients [14, 25, 26]. Conversely, recent studies could show an induction of the AMP hBD2 and 3 and RNase 7 in KC from both patients with AD and with psoriasis [27]. However, the overexpressed cytokines IL-4, -13 and -10 downregulate the TNF- α - and IFN- γ -

induced AMP expression in the skin of AD patients [14, 25, 26]. Both mobilization of hBD3 and killing of *S. aureus* are inhibited by IL-4 and IL-13, and neutralization of these cytokines has been shown to improve these activities [28]. Furthermore, IL-17-producing T cells, in particular Th2/IL-17 cells, have been shown to infiltrate acute AD reactions in atopy patch tests. IL-17 secretion is enhanced by the *S. aureus*-derived superantigen SEB. Although both healthy and AD KC upregulate hBD2 in response to IL-17, coexpression of IL-4 and IL-13 partially inhibited these effects. It has been assumed that the ineffective IL-17-dependent upregulation of hBD2 in patients with AD is due to a partial inhibition by the Th2-dominated microenvironment in acute AD [29].

In addition to the proneness to bacterial infections due to a deficiency of hBD2 and 3, the cathelicidin deficiency in AD predisposes to the development of severe viral infections such as the eczema vaccinatum and eczema herpeticum (EH) [25]. EH is a disseminated herpes simplex virus 1 or 2 infection with severe systemic illness that occurs in about 10–20% of patients with AD. Risk factors for EH are an early onset of AD, severe and untreated AD, head and neck dermatitis, previous herpes simplex infections and EH, an elevated serum IgE combined with a higher level of specific sensitizations, especially against *Malassezia sympodialis* [30]. In lesional skin of AD patients with EH, lower amounts of cathelicidin have been observed than in AD patients without EH, whereas IgE serum levels correlated inversely with the expression of LL-37 [25]. In both European and Afro-American patients with AD EH, a 3-fold higher frequency of the R501X *FLG* mutation has been found than in AD patients without EH, stressing the essential role of the skin barrier in the prevention of infections.

B-cell leukemia (Bcl)-3 acts as a transcriptional modulator of innate immune function in KC by modulating the expression of hBD3, cathelicidin, IL-8 and IL-5. Bcl-3 is inducible by the Th2 cytokines IL-4 and IL-13 and overexpressed in lesional skin of AD patients [31]. Silencing of Bcl-3 by small interfering RNA has been shown to reverse the downregulatory effect of IL-4 on hBD3 expression, indicating that Bcl-3 is required for IL-4-mediated suppression of hBD3. A vitamin D-responsive element has been identified in the promoter region of the human cathelicidin. Stimulation of KC with vitamin D₃ (1,25D₃) increased the transcription and activation of cathelicidin. Interestingly, Bcl-3 silencing enhanced 1,25D₃-induced cathelicidin expression in KC, suggesting a negative regulatory function in cathelicidin transcription. 1,25D₃ suppressed Bcl-3 expression in vitro and in vivo, suggest-

ing an autoregulatory role of 1,25D₃ in Bcl-3 function [31]. Furthermore, a downregulation of Bcl-3 expression [31] and upregulation of LL-37 in the skin of AD patients [32] could be shown after oral supplementation with vitamin D₃ in a small study conducted in adult AD patients as a promising new therapeutic option for AMP deficiency. However, further larger studies reproducing this effect are required.

Dermcidin (DCD), another antibacterial and antimycotic AMP, is constitutively expressed in human eccrine sweat glands and secreted into sweat. Patients with AD, most notably with a history of bacterial and viral infections, exhibit a significantly reduced amount of DCD-related peptides [33]. Additionally, the overall reduced amount of sweat in AD with a reduced secretion of IgA and an altered electrolytic composition of the sweat has been supposed to contribute to the susceptibility of AD patients to skin infections [33].

Furthermore, levels of the antimicrobial sphingolipid metabolite sphingosine are also decreased in the SC of AD patients. The sphingosine deficiency is supposed to result from decreased levels of ceramides as a substrate, and from diminished activities of its metabolic enzyme acid ceramidase, and it favors even further colonization of AD patients by *S. aureus* [34].

Thymic Stromal Lymphopoietin Functions as a Link between Disturbed Skin Barrier and Th2 Polarization in AD

KC are not only essential for the innate immune system due to the expression of TLR and production of AMP after exposure to microbes [35], but they also produce cytokines which mediate responses of the innate and adaptive immune system. KC of AD patients produce increased amounts of proinflammatory cytokines both constitutively as well as in response to several stimuli such as scratch-induced epidermal trauma, microbial infections and allergens. Most notably, the IL-7-like cytokine thymic stromal lymphopoietin (TSLP) is produced in high amounts by KC in AD, is upregulated by proinflammatory and Th2 cytokines [36] and functions as an important connector between disturbed skin barrier and Th2 polarization in AD. In immature CD11c⁺ DC, TSLP induces an increased expression of IL-4, -5 and -13 as well as of chemokines such as chemokine ligand (CCL)17 and CCL22, which leads to the recruitment of Th2 lymphocytes via binding of chemokine receptor (CCR)4. TSLP-activated CD11c⁺ DC then induce the expansion and dif-

ferentiation of naive CD8⁺ T cells into IL-5- and IL-13-producing effector cells which can exert cytotoxic effects via the production of IFN- γ in the presence of the CD40 ligand. Furthermore, TSLP has also been shown to activate mast cells and NK T (NKT) cells. Invariant NKT cells have been shown to be increased both among peripheral blood mononuclear cells and in the skin of patients with severe AD compared to those with mild or moderate AD and healthy controls. Invariant NKT cells have been shown to express the TSLP receptor and can be activated by TSLP to secrete IL-4 and IL-13 [37]. Transgenic mice with a skin-specific overexpression of TSLP develop an AD-like phenotype with eczematous lesions and inflammatory cellular dermal infiltrates, an increase in CD4⁺ Th2 cells with expression of homing receptors and increased IgE serum levels. Moreover, allergic skin inflammation elicited by epicutaneous immunization of mice with ovalbumin was severely impaired in TSLP receptor^{-/-} mice, which exhibited a decreased infiltration of eosinophils and decreased local expression of Th2 cytokines (IL-4, IL-13). Intradermal injection of anti-TSLP has been shown to block the development of allergic skin inflammation after cutaneous antigen challenge of ovalbumin-immunized wild-type mice [38], suggesting an essential role of TSLP in AD.

Chemokines Promote the Recruitment of Leukocytes into the Atopic Skin

Chemotactic cytokines (chemokines) are produced by KC, endothelial cells, leukocytes, macrophages, mast cells and DC and regulate the migration of T cells, monocytes, immature DC and eosinophils in extravascular tissues towards a chemical gradient with the help of various adhesion molecules. Increased levels of the chemokines CCL1/I-309, CCL2/monocyte chemoattractant protein-1, CCL3/macrophage inflammatory protein (MIP)-1 α , CCL4/MIP-1 β , CCL5/RANTES (regulated on activation, normal T cell expressed, and secreted), CCL11/eotaxin, CCL13/monocyte chemoattractant protein-4, CCL17/thymus and activation-regulated chemokine, CCL18/pulmonary and activation-regulated chemokine, CCL20/MIP-3 α , CCL22/macrophage-derived chemokine, CCL26/eotaxin-3, CCL27/cutaneous T-cell-attracting chemokine, CCL28/mucosa-associated epithelial chemokine and CX₃CL1/fractalkine as well as other chemotactic factors such as IL-16 and soluble CD30 have been found in the blood of AD patients compared to controls [39]. Enhanced CCL5 levels correlate to total

serum IgE levels and eosinophilia, whereas the serum levels of CCL11, 13, 26 and 28 [40] correlate directly with disease activity [39]. An increased expression of CCL11 and its receptor CCR3 has been found in lesional AD skin compared to control skin. In sequential skin biopsies of AD patients undergoing an atopy patch test, an upregulation of the mRNA expression of nearly all chemokines could be shown in response to the allergen exposition. The development of manifest eczema was mostly accompanied by an increased expression of CCL1, 17 and 18 as well as an increase in CCR5⁺ and CCR6⁺ inflammatory dendritic epidermal cells in the skin. Also, skin biopsies of chronic AD lesions feature a higher expression of CCL1, 17, 18 and 20 than do nonlesional AD skin and psoriasis. Thus, a specific contribution of these chemokines to AD has been supposed, whereas upregulation of many other chemokines has also been found in other chronic inflammatory skin diseases such as psoriasis [41]. The cytokine- and chemokine-mediated attraction of leukocytes induces the release of proinflammatory mediators and effector cytokines such as IL-31 and proteases (tryptase) which, together with stress-induced neuromediators, mediate pruritus [39]. Several studies stress the important role of CCL1, 2, 5, 13 and 20 and CX3CL1 in the recruitment of circulating progenitor cells of DC as well as of CCR3 ligands (CCL5, 11, 13 and 26) to eosinophils in atopic skin [39]. An induction of CCL18 and CCL1/CCR8 could be shown in vivo and in vitro by trigger factors of AD such as exposition to allergens and microbial antigens such as staphylococcal superantigens or *M. sympodialis*. Particularly CCL18, the skin-associated CCL27 and its receptor CCR10 appear to play a crucial role in the migration of T cells in inflammatory skin, the so-called T cell homing of activated CD4⁺ and cutaneous T cell antigen (CLA)-positive T cells [39]. As a result of continuous activation of epidermal cytokines due to the skin barrier dysfunction, already minimal exogenous skin trauma or exposure to other provocation factors such as microbial antigens or allergens suffice to amplify cytokine and chemokine production and to reactivate the disease in as yet clinically unaffected skin in AD patients [42].

***S. aureus* Aggravates AD by both Specific and Unspecific Mechanisms**

AD is frequently complicated by recurrent skin infections with bacterial, viral and mycotic pathogens. Most notably, *S. aureus* with its cell wall components LTA and

Table 1. Modified innate immune system in AD

Components	Main function	Findings in AD
Skin barrier	<ul style="list-style-type: none"> – physical barrier – prevents the entry of allergens, microbes and irritants into the skin – active immunological organ 	<ul style="list-style-type: none"> – ↑ transepidermal water loss – ↓ hydration of the skin – ↓ lipid content
CE	<ul style="list-style-type: none"> – prevents epidermal water loss – impedes the entry of allergens, irritants and infectious organisms – FLG degradation products such as hygroscopic amino acids serve as a natural moisturizing factor – acidic metabolites of FLG influence the pH of the skin and serine protease activity 	<ul style="list-style-type: none"> – ↓ expression of CE proteins: involucrin, loricrin, FLG, keratin K5 and K16 – <i>FLG</i> null mutations → ↓ production of FLG – <i>FLG</i> null mutations found in 16–23% of all AD patients (Central Europe), and in 22–45% (UK) increase the risk for eczema and AD with concomitant asthma – Th2 cytokines (IL-4 and IL-13) downregulate the expression of CE proteins FLG, loricrin and involucrin
Claudin 1	<ul style="list-style-type: none"> – tight junction protein controlling the epidermal permeability to water and other soluble components and immune cells 	<ul style="list-style-type: none"> – <i>CLDN1</i>-mutations – ↓ claudin 1 expression
SP	regulation of: <ul style="list-style-type: none"> – proteolytic degradation of the corneodesmosomes – desquamation of the corneocytes from the SC surface – proinflammatory effects via PAR2 	<ul style="list-style-type: none"> – mutations in the genes encoding SP SC chymotryptic enzyme, <i>KLK7</i> and <i>SPINK5</i> – ↑ SP activity by: – acute barrier disruption – slight, prolonged alkalinization in pH from 5.0 to 5.5 in AD patients
Ceramides	<ul style="list-style-type: none"> – lipid matrix as a critical barrier to transepidermal water loss 	<ul style="list-style-type: none"> – ↓ expression of ceramides in the skin – ↓ activity of acid sphingomyelinase-generating ceramides – ↑ secretion of ceramidase by <i>S. aureus</i>
KC	<ul style="list-style-type: none"> – epidermal cells/compound of the physical skin barrier – cytokine production 	<ul style="list-style-type: none"> – ↑ production of TSLP and other proinflammatory cytokines and chemokines constitutively and in response to several stimuli (scratch-induced epidermal trauma, microbial infections and allergens) → recruitment of Th2 cells, DC, eosinophils to the skin
PRR	<ul style="list-style-type: none"> – recognize highly conserved PAMP → microbial killing 	
TLR	<ul style="list-style-type: none"> – recognition of lipopolysaccharides (Gram-negative bacterial wall); unmethylated CpG nucleotides (bacterial DNA) 	<ul style="list-style-type: none"> – association of <i>TLR2</i> SNPs with severe forms of AD – modified inflammatory response dependent on <i>TLR2</i> polymorphisms – ↓ <i>TLR2</i> expression on macrophages of patients with AD → ↓ production of proinflammatory cytokines (IL-6, -8, -1β) of macrophages after stimulation with PGN and LTA – association of <i>TLR9</i> SNPs with intrinsic AD – lack of association with <i>TLR3</i>, 4 and 6, <i>IRAK-M</i> SNPs
NOD	<ul style="list-style-type: none"> – recognition of intracellular PAMP 	<ul style="list-style-type: none"> – association of <i>CARD4/NOD1</i>, <i>CARD15/NOD2</i> and <i>NALP12</i> SNPs with AD
MBL	<ul style="list-style-type: none"> – recognition of mannose-rich glycans, soluble PRR 	<ul style="list-style-type: none"> – association of <i>MBL</i> SNPs with AD in children, but not with AD severity
AMP	<ul style="list-style-type: none"> – microbial killing 	
AMP	<ul style="list-style-type: none"> – microbial killing 	<ul style="list-style-type: none"> – ↓ expression of cathelicidin LL-37, hBD2 and hBD3 – downregulation of AMP by the overexpressed cytokines IL-4, -13 and -10 – negative correlation of IgE levels with LL-37 expression – ineffective IL-17-dependent upregulation of hBD2 in AD due to a partial inhibition by the type 2 microenvironment
DCD	<ul style="list-style-type: none"> – antibacterial and antimycotic AMP 	<ul style="list-style-type: none"> – ↓ amount of DCD-related peptides
IgA	<ul style="list-style-type: none"> – mucosal immunity 	<ul style="list-style-type: none"> – ↓ amount of sweat in AD with ↓ IgA secretion

Table 1 (continued)

Components	Main function	Findings in AD
Bcl-3	– modulation of hBD3 and LL-37 expression in KC	– inducible by IL-4 and IL-13 and overexpressed in lesional AD skin – required for IL-4-mediated suppression of hBD3
Sphingosine	– antimicrobial sphingolipid metabolite	– ↓ sphingosine levels due to ↓ ceramide levels as a substrate; ↓ activities of its metabolic enzyme, acid ceramidase
Innate immune cells		
NK cells	– cell lysis and induction of apoptosis in virally infected cells and cells without expression of class I MHC molecules – secretion of proinflammatory cytokines	– preferential apoptosis of NK cells and $\gamma\delta$ + T cells in AD after contact with activated monocytes → ↓ circulating NK cells with a ↓ TNF- α and IFN- γ production
pDC	– production of antiviral IFN- α , - β – antigen-presenting cells	– ↑ number of pDC in peripheral blood – ↓ number of pDC in lesional AD skin – ↓ production of type I IFN after Fc ϵ RI preactivation
PMN	– phagocytic cell mediating acute antibacterial responses	– ↓ number of PMN in lesional AD skin – impaired phagocytosis and production of reactive oxygen species

AMP = Antimicrobial peptides; Bcl-3 = B-cell leukemia; CE = cornified envelope; DCD = demacidin; FLG = filaggrin; IFN = interferon; IL = interleukin; KC = keratinocytes; MBL = mannose-binding lectin; NK cells = natural killer cells; NOD = nucleotide-binding oligomerization domain; PAMP = pathogen-associated molecular pattern; pDC = plasmacytoid dendritic cells; PMN = polymorphonuclear leukocyte; PRR = pattern recognition receptor; SNPs = single nucleotide polymorphisms; SP = serine protease; TLR = Toll-like receptor; TSLP = thymic stromal lymphopoietin.

PGN is a current provocation factor for exacerbation of AD. A colonization by *S. aureus* of >90% could be shown in lesional, and of 76% in nonlesional skin of AD patients, while healthy skin has 5–30% *S. aureus* colonization. The increased secretion of ceramidase by *S. aureus* results in ceramide deficiency and, thus, contributes to the disturbed skin barrier in AD. An increased affinity and binding of *S. aureus* has partly been ascribed to the modified composition of fibrin and fibrinogen in AD [43]. *S. aureus* PGN stimulates the production of granulocyte macrophage colony-stimulating factor and CCL5 by KC [44], and thus adds to the recruitment of leukocytes and inflammation in AD. About 50–60% of the *S. aureus* strains found in AD produce enterotoxins such as *S. aureus* enterotoxin A (SEA), B (SEB), C (SEC), D (SED), etc., to which a part of the patients are sensitized. Moreover, enterotoxins can function as superantigens with antigen-independent T cell proliferation via direct interaction with the major histocompatibility complex (MHC) II complex and β -chain of the T cell receptor. Application of *S. aureus* or its enterotoxins has been shown to induce an eczematous inflammation. They lead to the recruitment of skin-homing CLA+ memory T cells and Langerhans cells in the atopic skin via the

induction of CCL1 and CCL18 production in DC, KC and endothelial cells [39]. SEB has been shown to enhance HDM-induced patch test reactions in patients with AD [45]. An induction of the pruritogenic cytokine IL-31 by staphylococcal superantigens could be shown both in vitro and in vivo. Superantigens have been assumed to suppress the suppressive functions of T_{reg} and to induce a resistance to corticosteroids via the production of glucocorticoid receptor- β [46]. The impact of these characteristics of *S. aureus* is clinically mirrored by the correlation of disease severity to colonization of eczematous lesions by *S. aureus* and to the amount of specific IgE levels of *S. aureus* enterotoxin. Moreover, *S. aureus* α -toxin has been shown to induce a Th1 response resulting in the proliferation of CD4+, INF- γ -producing T cells [47]. Furthermore, *S. aureus* has been shown to produce aureolysin, a metalloproteinase which cleaves and inactivates LL-37. Various *S. aureus* strains have been shown to express the *mprF* gene conferring resistance to defensins by modifying the charge on bacterial membranes [5]. Thus, *S. aureus* can promote its own survival by interfering with the AMP that are reduced anyway in AD.

Preferential Apoptosis of NK Cells and $\gamma\delta$ + T Cells, a Decreased Amount of IFN- α - and IFN- β -Producing Plasmacytoid DC and Impaired Recruitment of Polymorphonuclear Leukocytes Facilitate Infections in AD

NKT cells and type I IFN mediate the early innate immune response to viral infections. NK cells kill infected cells and cells that have lost expression of class I MHC molecules. NK cells have granules containing perforin, which creates pores in target cell membranes and granzymes which enter through target cell membranes and induce apoptosis of target cells, mainly cells infected with viruses and intracellular bacteria. NK cells also secrete IFN- γ , which activates macrophages to phagocytose and to kill microbes and secrete cytokines that stimulate inflammation.

AD patients have been shown to display profoundly reduced amounts of circulating NK cells and $\gamma\delta$ + T cells with a defective ability to sustain TNF- α and IFN- γ production after *in vitro* stimulation. Furthermore, apoptosis was preferentially observed in NK and $\gamma\delta$ + T cells from AD patients when stimulated in the presence of monocytes, and depletion of monocytes significantly protected these cells from apoptosis. It has been assumed that once NK and $\gamma\delta$ + T cells in AD patients are in immediate contact with activated monocytes, these cells are specifically targeted for apoptosis, leading to reduced type 1 cytokine production, thereby directing subsequent acquired immune responses toward a type 2 pattern and increasing susceptibility to infection [48].

Activated plasmacytoid DC (pDC) can produce the antiviral type I IFN- α and - β , which inhibit viral replication and enhance the recognition of MHC I-associated viral antigens on infected cells and, therefore, the efficiency of cytolytic T lymphocyte-mediated killing of these cells. pDC have been shown to express Fc ϵ RI, whereas the amount of Fc ϵ RI-bound IgE depends on disease activity and IgE serum levels [49]. Thus, pDC are able to process allergens via Fc ϵ RI-IgE and promote Th2 responses *in vitro*. While the number of pDC is increased in peripheral blood of AD patients, only a small number of pDC have been found in lesional epidermal skin of AD patients [50]. Aggregation of Fc ϵ RI on pDC induces the release of IL-10, and it could be shown *in vitro* that IL-10 as well as the overexpressed IL-4 induce apoptosis in a negative feedback loop [51]. Therefore, the influence of the Th2 microenvironment of the skin as well as a decreased expression of skin-homing molecules such as CLA and L-selectin CD62L in peripheral blood of AD patients have

been assumed to cause the low amount of pDC in atopic skin [51]. Fc ϵ RI-preactivated pDC produce lower amounts of type I IFN after stimulation with viral DNA and bacterial CpG [51], which together with the deficiency of AMP may contribute to the increased susceptibility of AD patients to infections.

Furthermore, an impaired recruitment of polymorphonuclear leukocytes (PMN) to lesional AD skin has been reported, which has been attributed to a decreased CD11b upregulation after activation by CXCL8/IL-8 and CXCL1/GRO α and priming (granulocyte macrophage colony-stimulating factor) stimuli as well as to a reduced production of PMN chemoattractants such as CXCL8/IL-8 and LL-37 in AD. Moreover, functional alterations in AD PMN with an impaired release of β -glucuronidase, leukotriene B $_4$, absent deposition of extracellular PMN granule proteins (lactoferrin and PMN elastase) in the skin, impaired phagocytosis and a reduced capacity to produce reactive oxygen species have been observed [5].

Conclusions

AD is often complicated by recurrent bacterial, viral and mycotic superinfections. Partly genetically based modifications of the innate immune system such as a disturbed skin barrier, modifications of PRR, deficiency of AMP, antiviral NK cells and pDC facilitate the entry of infectious microbes into the skin where they encounter immunocompetent cells. The Th2-driven microenvironment in the skin of AD patients further potentiates the dysfunction of the innate immune system, leading to a vicious circle promoting the disease (table 1.)

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