Keratinocytes: innate immune cells in atopic dermatitis

Short title: Keratinocytes and atopic dermatitis

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Abbreviations

AD: atopic dermatitis; **CCL**: CC chemokine ligand; **CE**: cornified cell envelope; **ΔNp63**: delta-N isoform of the transcription factor p63; **hBD**: human β-defensin; **hCAP18**: human 18-kDA cationic antimicrobial protein; **HDP**: host defense peptide; **IFN**: interferon; **IL**: interleukin; I**L-17Rh1**: IL-17 receptor homologue 1; **ILC**: innate lymphoid cell; **KLK**: kallikrein; **LPS**: lipopolysaccharide; **MCP**: monocyte chemotactic protein; **MDC**: macrophage-derived chemokine; **NLR**: nod-like receptor; **PARC**: pulmonary and activation-regulated chemokine; **PRR**: pattern recognition receptor; **RANTES**: regulated on activation, normal T-cell-expressed and secreted; **ST2**: suppression of tumorigenicity 2; **TARC**: thymus and activation-regulated chemokine; **Th**: T helper; **TLR**: Toll-like receptor; **TNF**: tumor necrosis factor; **TSLP**: thymic stromal lymphopoietin.

Abstract

The skin is a unique immune organ that constitutes a complex network of physical, chemical, and microbiological barriers against external insults. Keratinocytes are the most abundant cell type in the epidermis. These cells form the physical skin barrier and represent the first line of the host defense system by sensing pathogens via innate immune receptors, initiating antimicrobial responses and producing various cytokines, chemokines and antimicrobial peptides, which are important events in immunity. A damaged epidermal barrier in atopic dermatitis allows the penetration of potential allergens and pathogens to activate keratinocytes. Among the dysregulation of immune responses in atopic dermatitis, activated keratinocytes play a role in several biological processes that contribute to the pathogenesis of atopic dermatitis. In this review, we summarize the current understanding of the innate immune functions of keratinocytes in the pathogenesis of atopic dermatitis, with a special emphasis on skin-derived antimicrobial peptides and atopic dermatitis-related cytokines and chemokines in keratinocytes. An improved understanding of the innate immunity mediated by keratinocytes can provide helpful insight into the pathophysiological processes of atopic dermatitis and support new therapeutic efforts.

Introduction

Atopic dermatitis (AD) is one of the most frequent chronic and recurrent inflammatory skin conditions, and it is characterized by intense itching at different stages of eczematous lesions [1]. One hallmark of AD is an accumulation of inflammatory cells, including T cells, macrophages, eosinophils and mast cells, in lesional tissue [1]. Histopathological examination of nonlesional atopic skin reveals a sparse perivascular T-cell infiltrate in the dermis, which suggests that immune dysregulation is a key defect in AD [1]. During the past decade, significant progress was made in our understanding of immune dysregulation in AD. The contribution of different T-cell subsets and a complex interplay between inflammatory cells and epidermal keratinocytes emerged as critical components in disease pathogenesis.

Epidermal keratinocytes are the prominent cell type in human skin. These cells are essential for permeability barrier formation and provide the mechanical strength of the skin. As the first line of defense against harmful external invaders, keratinocytes express different pattern recognition receptors (PRRs) on their cell surfaces that allow these cells to recognize various pathogens and initiate immune responses. Keratinocytes secrete a range of effector molecules, including cytokines, chemokines, antimicrobial peptides (host defense peptides (HDPs)), growth factors and lipid mediators, which contribute to

the recruitment of inflammatory cells. Keratinocytes play a significant role in the regulation of skin immune homeostasis. Therefore, an imbalance in keratinocyte activities is associated with development and progression of AD. Herein, we summarize the recent progress in understanding the immunological barrier functions of keratinocytes and the roles of these cells in the pathogenesis of AD.

Overview of keratinocyte biology

The skin provides several types of defensive functions, including physical, thermal, permeability and an immune barrier to protect the integrity of human beings. The epidermis is responsible for the primary barrier function of the skin. A keratinized stratified squamous epithelium is composed of cells called keratinocytes. These cells undergo a process of epidermal differentiation or keratinization from the basal layer to differentiating spinous, granular, and cornified layers [2]. The keratinocytes in each layer have unique morphological and biochemical features and are under the control of a highly complicated and tightly regulated process. Several transcription factors, including transcription factors p63, AP-2γ, Notch and the Wnt signaling pathways play key roles in controlling keratinization via influencing the proliferation, differentiation and survival of keratinocytes [2]. The transition of proliferating basal keratinocytes to differentiating keratinocytes is associated with a switch in expression from basal keratin 5 and keratin 10 to suprabasal keratins (keratin 1, 2 and 10), which are markers of keratinocyte differentiation [3]. Other keratins are expressed in a specific body site or a cell-specific manner. For example, keratin 9 is specifically expressed in the suprabasal layer of the stress-bearing epidermis, and keratins 6, 16 and 17 are expressed in hyperproliferative or regenerating epidermis [3].

The expression of a marker for late keratinocyte differentiation, profilaggrin, and its further degradation products is a calcium-dependent process [4]. Profilaggrin is the main protein of keratohyalin granules, which are located within keratinocytes of the granular layer [4]. After the proteolytic processing of profilaggrin, mature filaggrin is further degraded into free amino acids, trans-urocanic acid and pyrrolidone-5-carboxylic acid, which are components of a natural moisturizing factor [4]. Filaggrin promotes the aggregation of keratin filaments into tight bundles, which facilitate the flattening of keratinocytes. The highly insoluble cornified cell envelope (CE) precursor proteins, including envoplakin, periplakin, involucrin, loricrin and small proline-rich proteins, are polymerized and cross-linked via keratinocyte-specific transglutaminases in a calcium-dependent manner to form the CE at the inner surface of the cell membrane [5]. Therefore, terminally differentiated keratinocytes consist of tightly packed keratin filaments that are covalently attached to the CE, which provides a permeable barrier to prevent transcutaneous water loss and the entry of harmful substances.

Intercellular adhesion complexes are important regulators of the epidermal integrity and prevent the entrance of harmful substances and pathogenic microorganisms by tightly sealing the paracellular space between adjacent keratinocytes [6]. The intercellular adhesion complexes are composed of desmosomes, adherens junctions and tight junctions

[6]. Desmosomes and adherens junctions provide attachment sites for the keratin filaments and actin filaments, respectively, which hold the keratinocytes together and contribute to the mechanical strength and structural continuity of the entire epidermis [6]. In contrast, tight junctions are specifically restricted to the apical borders of keratinocytes in the granular layer. The major components of tight junctions include the claudins, occludin and junction adhesion molecules [6,7]. Tight junctions limit the passage of substances and are crucial in the regulation of keratinocyte proliferation and differentiation [7].

During keratinization, the epidermal lipid composition also changes extensively. Specialized lipid-rich organelles called lamellar bodies are responsible for the epidermal lipid in cutaneous barrier homeostasis. Lamellar bodies are found in the keratinocytes of the upper spinous and granular layers. These bodies consist of closely packed stacks of numerous dense membrane-bound sacs that are rich in lipids, including the glucosylceramides, sphingomyelin, phospholipids and cholesterol, and contain several lipid-processing enzymes, such as glucocerebrosidase, sphingomyelinase and phospholipase A [8]. The contents are extruded into the stratum granulosum/stratum corneum interface, and the lipid precursors undergo hydrolysis and enzymatic conversion into ceremide, free fatty acids and cholesterol to form intercellular lipid sheets [8]. An

overview of epidermal differentiation is shown in Fig. 1. Skin-derived HDPs, such as cathelicidin LL-37 and human β-defensins (hBDs), are stored in the lamellar bodies until release [9,10]. Therefore, the secreted lamellar body contents are required for normal epidermal permeability functions and antimicrobial defense mechanisms.

Keratinocytes provide skin structure and play roles in the initiation and modulation of host immune responses via recognition of danger signals and the production of various cytokines, chemokines and HDPs. As first responders, the keratinocytes express various PRRs, including Toll-like receptors (TLRs), nod-like receptors (NLRs) and C-type lectin receptors [11]. These receptors recognize highly conserved structure components of microorganisms, known as pathogen- or damage-associated molecular patterns. Different PRRs work in concert in the promotion of potent innate immune responses against microbes. Functional expression in keratinocytes was shown for extracellular TLRs (TLR1, 2, 4, 5 and 6) and intracellular TLRs (TLR3 and 9) following stimulation by their corresponding TLR-specific ligands [12]. Briefly, TLR2, together with TLR1 or TLR6, recognize motifs of Gram-positive bacteria, such as peptidoglycan and lipoteichoic acid [12]. Lipopolysaccharides (LPS) and bacterial flagellin activate TLR4 and TLR5 on keratinocytes, respectively [12]. Viral recognition via the intracellular receptors TLR3 and TLR9 stimulates interferon (IFN)-mediated antiviral responses [12]. C-type lectin

receptors on keratinocytes recognize fungal surface components [11], and NLRs are activated by both exogenous danger signals, such as ultraviolet B, and endogenous danger signals, including cancer [13,14].

Although PRRs recognize products of pathogenic and commensal bacteria, several commensal microorganisms do not normally trigger a pathogenic inflammatory response. The well-established skin flora *Staphylococcus epidermidis* activates a protective immune response via the induction of hBD production and suppresses the TLR3-mediated inflammatory response via activation of TLR2 on keratinocytes [9,15], which supports evidence that skin commensals are critical contributors to the maintenance of immune homeostasis [16]. However, patients with AD have notable differences in skin microbial community composition, including alterations in commensal microorganisms and the presence of pathogenic bacteria [16]. Lesional and nonlesional atopic skin are almost exclusively colonized with *Staphylococcus aureus* (*S. aureus*) [16]. The abundance of *S. aureus* correlates with disease severity [16].

In response to immunological and inflammatory stimuli, keratinocytes produce different sets of immune mediators that coordinate between keratinocytes and sentinel skin-resident immune cells, including T cells, mast cells, macrophages and specialized dendritic cells called Langerhans cells [11]. Keratinocytes and immune cells determine

the appropriate adaptive immune responses. The antigen presenting cells recognize and take up foreign proteins then transport the proteins to the draining lymph nodes for priming of the appropriate immune responses. Aberrations in the interaction between resident keratinocytes and T cells may activate inflammation and the immune circuits responsible for the initiation, progression and persistence of inflammatory skin diseases, such as AD.

Keratinocytes as a potent initiator of the inflammatory cascade in AD

A defective permeability barrier is of prime importance in the pathogenesis of AD. Lesional and nonlesional AD skin show decreased stratum corneum hydration and an abnormal expression of epidermal terminal differentiation markers, including involucrin, loricrin and filaggrin [17,18]. The genes encoding these proteins are downregulated in AD compared to normal subjects [17,18]. Filaggrin loss-of-function mutations are associated with elevated serum immunoglobulin E levels and allergic sensitizations in AD [17,18]. Dysregulation of the interleukin (IL)-1 axis may account for the initiation of inflammatory responses in AD [19]. An upregulated expression of proinflammatory cytokines, including IL-1β and IL-18, was observed in AD patients with filaggrin mutation. These cytokines promote the chemotaxis of inflammatory cells and alter the activity of multiple serine proteases, which results in further barrier disruption in AD [19].

Danger signals from barrier disruption and microbial invasion trigger the production of various keratinocyte-derived cytokines, such as IL-6, IL-18, IL-23, granulocyte-macrophage colony-stimulating factor and tumor necrosis factor (TNF)-α, which exhibit proinflammatory activities and promote the activation, differentiation and recruitment of inflammatory cells to the skin in AD [20-23]. The combination of IL-1β, IL-6 and transforming growth factor-β promote T helper (Th) 17 cell activation, which plays an important part in the early stage of AD [20]. Increased IL-17 expression is associated with acute skin inflammation in AD [20]. IL-17 induces the production of pro-inflammatory cytokines and stimulates Th2 cells to produce IL-4, which likely contribute to the initiation of AD inflammation [21,22].

Keratinocytes are the primary source of the chemokines that recruit inflammatory cells, such as dendritic cells, mast cells, eosinophils and T cells, into AD skin [21,23]. The increased production of diverse chemokines, such as eotaxin/CC chemokine ligand (CCL) 11, monocyte chemotactic protein (MCP)-1/CCL2, MCP-4/CCL13, pulmonary and activation-regulated chemokine (PARC)/CCL18, and thymus and activation-regulated chemokine (TARC)/CCL17, was demonstrated in lesional sites of AD [23,24]. Elevated serum levels of eotaxin-3/CCL26 and TARC/CCL17 also correlate with disease activity in AD [23]. RANTES (regulated on activation, normal T-cell-expressed and

secreted) is a potent chemokine in allergic inflammation, and it is higher in skin lesions with severe AD than mild AD [25]. The chemokine receptor CCR3 binds RANTES/CCL5, eotaxin/CCL11, eotaxin-3/CCL26 and MCP-4/CCL13, and it is expressed selectively on eosinophils, basophils and Th2 cells, which leads to amplification of Th2-cell recruitment and allergic response in AD [23,24]. Table 1 summarizes the keratinocyte-derived cytokines and chemokines that are involved in the pathogenesis of AD.

Keratinocyte-derived cytokines regulate Th2 immunity

Keratinocyte-derived cytokines regulate effector T-cell differentiation in AD [21,23]. Barrier disruption stimulates keratinocytes to release Th2-promoting cytokines, including thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 [26]. Keratinocytes and other epithelial cells at barrier surfaces in subjects with atopic diseases constitutively expressed these early signals, which act on innate effector cells, such as dendritic cells, mast cells, basophils and type-2 innate lymphoid cells (ILC2s) [26]. As mentioned above, various transcription factors are implicated in keratinocyte activation. The overexpression of the delta-N isoform of the transcription factor p63 (ΔNp63) in transgenic mouse epidermis produced epidermal hyperplasia, defective epidermal differentiation, and Th2 cell-mediated immunological changes that closely resembled human AD lesional skin [27].

ΔNp63 also plays a role in mediating the itch response via regulation of the expression of IL-31, IL-33 and TSLP, which are key mediators of pruritic eczema in AD [27]. Other studies revealed the roles of the Notch signaling pathway in the regulation of inflammation-related itch in AD [28]. A downregulation of Notch receptors is found in lesional skin of patients with AD [28]. Disruption of Notch signaling in mice causes a disturbance of the epidermal barrier and accelerates inflammatory dermal infiltration, with increased expression of TSLP [28]. Therefore, transcriptional regulators of keratinocyte development and differentiation may be involved in the pathogenesis of AD.

TSLP is an epithelial cell-derived IL-7-like cytokine that is released in response to mechanical injury, microbial infection and allergen exposure [26,29]. Increased expression of TSLP was observed in filaggrin knockdown keratinocytes after TLR3 ligand stimulation [30]. The role of TLR activation in TSLP overexpression is not confined to TLR3 activation. Engagement of TLR5 and TLR2/6 by their cognate ligands also causes an upregulation of TSLP [31]. Enhanced enzymatic activity of kallikrein (KLK) 5 drives the production of TSLP via G protein-coupled protease-activated receptor 2 [21,29,32]. The expression of TSLP in AD skin correlates with the severity of the disease and the degree of epidermal barrier disruption [29]. TSLP directly activates dendritic cells to polarize naïve T cells toward Th2 cells that secrete IL-4, IL-5 and IL-

13, which further induce TSLP release [26,31]. TSLP inhibits the production of HDPs, including hBD-2, by keratinocytes, which correlates with the susceptibility of the skin to infections [9]. In contrast, activation of TSLP enhances the neutrophilic killing function that is an essential host defense mechanism against skin bacterial infection caused by methicillin-resistant *S. aureus* and *Streptococcus pyogenses* [33]. TSLP has a nonimmunological effect as a pruritogen and promotes robust scratching behaviors. Keratinocyte-derived TSLP acts directly via TSLP receptors located on cutaneous sensory neurons and contributes to pruritus induction [34]. Recent clinical trial data demonstrated improvement in atopic itch and the severity of associated atopic conditions after treatment of patients with a monoclonal anti-TSLP antibody [26,35].

IL-25 and IL-33 are also key keratinocyte-derived mediators of the allergic reaction and initiate the development of the Th2 response [26,36]. IL-25 (IL-17E) is a member of the IL-17 family, and it shares structural similarity with other IL-17 members [36]. IL-25 signals via IL-25 receptors, which are composed of the IL-17B receptor, named IL-17 receptor homologue 1 (IL-17Rh1), and Evi27 [36,37]. A recent immunohistochemical study of IL-25 expression in the skin of patients with AD revealed an increased number of IL-25- and IL-17Rh1-expressing cells in lesional and nonlesional AD skin compared to skin from healthy individuals [38]. Keratinocytes constitute the majority of IL-25-

expressing cells in AD skin [38]. Allergen-activated mast cells, eosinophils, basophils and dermal dendritic cells also secrete IL-25 [36,38]. An increased number of IL-25-expressing dendritic cells was also detected after LPS stimulation [38]. Keratinocyte-derived IL-25 is involved in the induction of Th2 cells via direct activation of naïve $CD4^+$ T cells or ILC2 activation to promote innate type-2 immune responses [36]. IL-13 production in activated ILC2 consequently promotes TARC/CCL17- and macrophage-derived chemokine (MDC)/CCL22-mediated recruitment of Th2 cells in AD lesional skin [39]. Unlike other IL-17 cytokine members, IL-25 suppresses cell-mediated immunity by preventing the development of the Th1 or Th17 immune response [36]. Elevated levels of IL-25 in atopic lesional skin suppress the synthesis of filaggrin by keratinocytes, which compromises the skin barrier function [38].

IL-33 is a pro-inflammatory cytokine in the IL-1 family that is closely related to IL-1β and IL-18 in structure [40]. IL-33 is produced by endothelial cells and various epithelial cells, including keratinocytes, constitutively express IL-33 as an inactive precursor [40]. In response to infection or tissue injury, the IL-33 precursor is immediately cleaved by caspase-1 to form an active secreted IL-33 [40]. Subsequently, active IL-33 activates mast cells, basophils and ILC2 to secrete IL-4, IL-5 and IL-13 via the receptor suppression of tumorigenicity 2 (ST2) [26,40]. In addition to triggering Th2 polarization, IL-33 promotes

the secretion of pruritic cytokines, including TSLP and IL-31, from keratinocytes and Th2 cells, respectively, which amplifies Th2 responses [26]. Similar to TSLP, IL-33 mediates the itch response via the activation of itch-sensing sensory neurons [40]. IL-33 directly disrupts epidermal barrier function via the downregulation of filaggrin expression and the tight junction protein expression of claudin-1 [40-42]. Therapeutic strategies to inhibit IL-33 or ST2 receptor activities are under investigation as treatments for moderate to severe AD. An anti-IL-33 monoclonal antibody Etokimab was used in recent phase II clinical trials, which found beneficial effects in AD with an associated substantial reduction in skin inflammatory cascades [26,43].

Altered keratinocyte-derived HDP expression in AD

In addition to the production of various cytokines and chemokines, keratinocytes are a primary source of HDPs. Differentiating keratinocytes in the epidermis express protective skin-derived HDPs, such as cathelicidin LL-37, hBDs, S100A7 and RNase 7 [44]. These peptides exhibit broad-spectrum antimicrobial activities against commonly found skin pathogens, pro- and anti-inflammatory properties and immunomodulatory activities [44]. The immunomodulatory functions of HDPs include chemoattractant activity, the induction of cytokine and chemokine production and promotion of cell proliferation and migration [44]. HDPs also possess beneficial effects on the maintenance of epithelial

barrier function via the regulation of transepidermal water loss [45] and induction of the localization and distribution of tight junction proteins, which leads to improvement of tight junction barrier function [44].

LL-37 is the only human member of the cathelicidin HDP family [10]. The precursor protein of LL-37 is hCAP18 (human 18-kDA cationic antimicrobial protein) and localized in the lamellar bodies of keratinizing cells [10]. hCAP18 is cleaved via a proteolytic process of epidermal serine proteases, such as KLK5 and KLK7, to form the active peptide LL-37, which is released into the intercellular spaces and creates a protective barrier against infectious agents [10]. Several resident and infiltrating immune cells, including Langerhans cells, dendritic cells, neutrophils and mast cells, synthesize and release LL-37 in response to inflammatory stimuli [10]. In addition to the antimicrobial activity against various pathogens that colonize atopic skin, LL-37 may be useful in the treatment of AD. For example, LL-37 upregulates the nerve repulsion factor semaphorin 3A (which is reduced in AD) [46] and suppresses nerve elongation factors, including nerve growth factor [47]. LL-37 may restore the impaired skin barrier in AD patients via its ability to induce the distribution of tight junction proteins in keratinocytes [48]. The finding that mice deficient in a mouse homolog to LL-37, the cathelin-related antimicrobial peptide, exhibit a delay in the recovery of the permeability barrier following

acute perturbation further demonstrates the role of LL-37 in the maintenance of skin barrier homeostasis [45]. LL-37 exhibits pro- and anti-inflammatory effects, and it is involved in keratinocyte differentiation [10]. The immunomodulatory effects of LL-37 may contribute to the pathogenesis of AD via its ability to exert chemotaxis of mast cells, promote histamine production and induce chemokine receptor expression CCR2 and CXCR4, which are expressed on the cell surface of eosinophils [10,23,49,50].

hBDs are also key players in keratinocyte-driven immunity. hBD-1 is constitutively expressed in suprabasal keratinocytes, sweat glands and the sebaceous glands, and hBD-2, hBD-3 and hBD-4 are induced via stimuli, such as microbial pathogens and pro-inflammatory cytokines, such as IL-1β, IL-17, IL-22 and TNF-α [9]. hBDs are expressed in the lamellar bodies of the spinous layer and the intercellular space of the upper epidermis in a pattern that is similar to LL-37 [9]. The potent antimicrobial activities of hBDs likely vary depending on their cationicity [44]. hBD-3 demonstrates the strongest antimicrobial activity against *S. aureus*, herpes simplex virus and *Candida* species [9]. Notably, these microbial pathogens are abundant on AD skin and associated with immune dysregulation and disruption of the skin barrier in AD [17]. hBD-3 exhibits powerful antimicrobial activity and enhances the skin barrier function via the induction of the

keratinocyte tight junction proteins involved in maintaining permeability barrier homeostasis [9,51].

Although the expression of HDPs is greatly increased during infection, inflammation and injury, LL-37 and hBDs are altered in atopic individuals [9,44]. The expression of LL-37, hBD-2 and hBD-3 is lower in AD lesions compared to psoriatic skin lesions, and this downregulation affects the susceptibility to skin infection in AD patients [9,44,52,53]. The reduced levels of HDPs may be explained by the predominance of Th2-derived cytokines, which act as strong inhibitors of LL-37, hBD-2 and hBD-3 production [9,44,53-55]. The pruritic cytokines, including IL-31 and TSLP, also have inhibitory effects on the production of these HDPs [9]. Defects in the lamellar body secretory system in AD lead to an aberrant expression of KLKs with increased enzymatic activities, which accelerates the proteolytic degradation of LL-37 [10]. Filaggrin mutation in AD skin promotes perturbation of the skin barrier and affects the lamellar body secretory system, but the impact of filaggrin on HDP expression is controversial [9].

Although the expression of HDPs is generally low in AD skin compared to psoriasis, increased levels of LL-37 and hBDs were reported in lesional AD skin compared to healthy controls [9,54]. The role of IL-17 and IL-22 in AD emerged recently, and both cytokines are potent inducers of LL-37 and hBDs, which may partially explain the

upregulation of HDPs in lesional AD skin compared to normal subjects [9,58-60]. Skin damage following excessive scratching in AD may underlie the increase in HDP expression in inflammatory atopic skin [9].

S100A7 and RNase 7 are induced in AD lesional skin [44,56,57]. S100A7, or psoriasin, belongs to the S100 protein family, which is a low molecular weight protein family that is characterized by the presence of EF-hand calcium-binding proteins [56]. Their encoding genes are located within the epidermal differentiation complex that encodes many keratinocyte differentiation proteins, such as loricrin, involucrin and filaggrin [56]. Keratinocytes are a major source of S100A7 in human skin, and its expression increased following stimulation with inflammatory cytokines, growth factors, flagellin and vitamin D [56,58]. S100A7 promotes leukocyte chemotaxis and induces chemokine secretion, the production of reactive oxygen species and the release of α-defensins from neutrophils [59]. S100A7 is also implicated in a number of skin inflammatory disorders, including psoriasis, acne vulgaris, squamous cell carcinoma and AD [44,56]. S100A7 preferentially and very effectively controls the growth of *Escherichia coli*, which explains why this bacterium rarely colonizes normal skin and AD skin [60].

The abundant expression of RNase 7 is induced in keratinocytes of inflammatory skin disorders, including AD [57]. Numerous microbial elements and various inflammatory

mediators, such as IL-1β and IL-17, contribute to the regulation of RNase 7, which exhibits potential activity against *S. aureus*. [57]. RNase 7 plays an important role in the host defense against infectious pathogens, and it inhibits Th2 cytokine production, which prevents Th2-mediated allergic responses [57]. However, IL-31 prevents the expression of RNase 7 during the host defense against *S. aureus* [57], which illustrates the complex interplay between the innate and adaptive immunity in the regulation of HDPs in AD.

Keratinocytes in the allergic inflammatory cascade

AD is generally known as a Th2-driven disease. The ability of Th2 cytokines to regulate the course of AD is a direct effect on keratinocytes. IL-4 and IL-13 expression are increased in acute eczematous skin lesions and uninvolved skin of AD patients [1]. Keratinocytes constitutively express functional IL-4 and IL-13 receptors [61] and produce the eosinophil chemokine eotaxin-3/CCL26 in response to IL-4 and IL-13 [18,23]. IL-4 substantially induces a proinflammatory response in keratinocytes via potentiating IFN-γ and TNF-α in the induction of CXCR3 agonistic chemokines, such as monokine induced by IFN-γ/CXCL9, IFN-γ-induced protein 10/CXCL10 and IFN-inducible T-cell α-chemoattractant/CXCL11, which recruits more T cells into the inflamed skin [62]. IL-4 and IL-13 attenuate the expression of keratinocyte structural components, including keratin 1, keratin 10, filaggrin, involucrin, and loricrin, which leads to a worsening of

barrier dysfunction [61,63]. IL-4 and IL-13 also increase the protease activity of KLK7 in epidermal keratinocytes, which triggers skin desquamation via digestion of desmosomal proteins [61]. Ceramide is a major lipid component of lamellar bodies, and the IL-4-induced depletion of ceramide in AD skin leads to profound alterations of barrier function, which results in xerosis skin [61].

IL-4 participates in allergic inflammation, defective skin barrier and bacterial colonization of AD skin. IL-4 enhanced the expression of adhesion molecules for *S. aureus*, including fibronectin and fibrinogen [64,65]. Microbial biofilms produced by *S. aureus* also play important pathogenic roles in the initiation and development of AD via regulation of inflammatory cytokine production in keratinocytes [16]. Biofilm-induced IL-6 production reduces the expression of keratin 1, keratin 10 and filaggrin, which affects keratinocyte differentiation and leads to an increased susceptibility to bacterial infections [16,66]. The staphylococcal biofilm and superantigen play roles in Th2 polarization and the pathogenesis of pruritus via the induction of IL-31 expression in atopic individuals [16].

The novel Th2 cytokine IL-31 is a critical cytokine in AD. Increased expression of IL-31 occurs in pruritic AD lesions [67]. Th2 cells and mast cells primarily produce IL-31 following stimulation with allergen, staphylococcal superantigen and reactive oxygen

species [67]. Keratinocytes from AD patients express high levels of IL-31 receptors and respond to IL-31 to produce various atopy-associated chemokines, such as TARC/CCL17 and MDC/CCL22 [23,67-69]. A coculture of IL-31-activated eosinophils with keratinocytes resulted in the release of inflammatory cytokines/chemokines, including IL-1β, IL-6, MCP-1/CCL2 and PARC/CCL18 [70]. IL-31 also downregulates filaggrin expression in keratinocytes, and it is implicated in itch induction via its receptor on cutaneous nerve fibers in lesional skin [67,71,72]. The persistent scratching in AD causes chronic inflammation and may lead to secondary infection [1]. Notably, the observation that LL-37 and hBDs increase the production of IL-31 and other pruritogenic factors suggests a harmful role of HDPs in the development of AD [73].

Histamine is a well-known pruritogenic mediator released by mast cells. The histamine H1 receptor is present on the surface of pruriceptive neurons, and it is predominately expressed by human keratinocytes [74]. Histamine stimulates the inflammatory cytokine cascade and downregulates the expression of keratinocyte differentiation-associated proteins keratin 1, keratin 10, filaggrin and loricrin, and it inhibits the expression of tight junction barrier proteins, which leads to barrier dysfunction during skin inflammation [74,75]. In contrast, histamine contributes to innate immunity via the induction of hBD-2 and hBD-3 production from keratinocytes [9].

Th2 cytokines are predominant in the acute phase of AD, but IL-22 was implicated in the chronic phase of AD when the epidermis becomes thickened due to the hyperplasia of keratinocytes (acanthosis) [1,76]. IL-22 is a member of the IL-10 cytokine family, and it is secreted by different types of T cells, including Th17 and Th22 cells [77]. Pronounced infiltration of Th22 cells and increased expression of IL-22 in skin lesions of patients with AD have been reported [77,78]. IL-22 has multiple effects on keratinocytes, including the promotion of keratinocyte proliferation and migration via STAT3 phosphorylation, which is downstream of the IL-22 receptor in keratinocytes [79]. IL-22 also compromises skin barrier function via the downregulation of the expression of filaggrin, claudin and epidermal transglutaminase 3 [80]. Activated Th22 cells induce the production of several cytokines and chemokines, including pruritogenic mediators (TSLP and IL-33), and contribute to chronic inflammation in atopic skin [80]. Beneficial effects of an anti-IL-22 monoclonal antibody (fezakinumab) in moderate to severe AD, including a profound reduction in disease activity, were reported in a phase II clinical trial [81].

Conclusion

As part of the innate immune system, keratinocytes initiate crosstalk between innate and adaptive immune responses by regulating the release of several key molecules that trigger inflammatory reactions and immune responses in AD (Fig. 2). An understanding of the processes underlying AD should help in formulating appropriate therapies to improve AD patient quality of life and prevent AD-related allergic disorders.

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Conflict of interest

The authors declare no conflicts of interest.

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Figure legends

Figure 1. Epidermal keratinocyte differentiation

During the process of epidermal differentiation, keratinocytes synthesize vital components that are necessary for epidermal barrier function. The expression patterns of keratin intermediate filaments and transglutaminases correlate with the stage of epidermal differentiation. The intercellular adhesion complexes, including desmosomes and tight junction, provide strong mechanical attachments between adjacent keratinocytes. Lamellar bodies are secretory organelles containing lipids and enzymes that contribute to the formation of the epidermal lipid barrier. Keratohyalin granules are expressed in the stratum granulosum and contain profilaggrin, which is the precursor of filaggrin, which facilitate the aggregation of keratin filaments, and loricrin is a major component of the cornified cell envelope.

Figure 2. The contribution of keratinocyte-derived mediators to the pathogenesis of atopic dermatitis

An impaired epidermal barrier in atopic dermatitis (AD) allows for the penetration of potential allergens, irritants and pathogens, such as *Staphylococcus aureus*, to initiate the

immune activation of keratinocytes. Activated keratinocytes produce a plethora of proinflammatory cytokines and chemokines, including eotaxin/CC chemokine ligand (CCL) 11, eotaxin-3/CCL26, monocyte chemotactic protein (MCP)-4/CCL13, regulated on activation, normal T-cell-expressed and secreted (RANTES)/CCL5, and thymus and activation-regulated chemokine (TARC)/CCL17, which attract and activate Langerhans cells (LCs), dendritic cells (DCs), eosinophils, basophils, mast cells, type-2 innate lymphoid cells (ILC2) and T helper (Th) 17 cells. The cytokines released by these cells induce Th2 polarization, which plays a key role in the pathogenesis of AD. Keratinocytes also produce Th2-promoting cytokines, including thymic stromal lymphopoietin (TSLP), interleukin (IL)-25 and IL-33, which further amplify the Th2 immune responses and exacerbate AD by inducing itching. TSLP, IL-33 and Th2 cytokines (IL-4, IL-13 and IL-31) inhibit the expression of skin-derived host defense peptides (HDPs), and increased expression of IL-22 upregulates HDP production in the chronic stages of AD. HDPs, including LL-37, human β-defensins (hBDs), S100A7 and RNase 7 improve the skin barrier integrity and promote antimicrobial barrier function in AD skin. GM-CSF, granulocyte-macrophage colony-stimulating factor; Ig, immunoglobulin; TNF, tumor necrosis factor.

Table 1. The keratinocyte-derived cytokines/chemokines associated with AD pathogenesis

Cytokine/ chemokine	Receptor	Up-regulation	Target cell	Effect	Reference
CCL1 (I-309)	CCR8	Allergens, molluscum contagiosum, *S. aureus*	DCs, LCs, T cells	Chemoattractant for DCs, LCs and memory T cells	[23,24]
CCL2 (MCP-1)	CCR2	IFN-γ	DCs, monocytes, T cells	Chemoattractant for DCs, monocytes and T cells	[23,24]
CCL5 (RANTES)	CCR1, CCR3, CCR5	IL-4, IFN-γ, TNF-α	Basophils, DCs, eosinophils, T cells, fibroblasts, keratinocytes	Chemoattractant for DCs, eosinophils, monocytes and T cells	[23-25]
CCL11 (Eotaxin)	CCR3	IL-4, IL-13, IFN-γ	Basophils, DCs, eosinophils, T cells	Chemoattractant for eosinophils	[23,24]
CCL13 (MCP-4)	CCR2, CCR3	IL-1, IL-4, IFN-γ, TNF-α	Basophils, DCs, eosinophils, monocytes, T cells	Chemoattractant for DCs, eosinophils, basophils and T cells	[23,24]
CCL17 (TARC)	CCR4	IL-31, TNF-α, IFN-γ	NK cells, T cells	Chemoattractant for Th2 cells	[23,24,67]
CCL18 (PARC)	CCR8	Allergens, staphylococcal superantigens	T cells	Chemoattractant for LCs and memory T cells	[23,24]
CCL20 (MIP-3α)	CCR6	TGF-β	T cells, DCs	Chemoattractant for DCs and memory T cells	[23,24]
CCL22 (MDC)	CCR4	IL-13, IL-31	T cells	Chemoattractant for Th2 cells	[67]
CCL26 (Eotaxin-3)	CCR3	IL-4, IL-13, IFN-γ	Eosinophils, T cells	Chemoattractant for eosinophils and T cells	[23,24]

Table 1. ***Continued***

Cytokine/ chemokine	Receptor	Up-regulation	Target cell	Effect	Reference
CCL27 (CTACK)	CCR10	IL-1β, TNF-α	T cells	Chemoattractant for memory T cells	[23,24]
IL-1β	IL-1RI, IL-1RAcP	TLR4 ligands, skin barrier disruption	T cells, endothelial cells, fibroblasts	Proinflammatory cytokines; induction of T cell activation and basophil histamine release	[19,20,23, 82]
IL-1RA	IL-1R1	IL-4, IL-13	T cells, endothelial cells, fibroblasts	Anti-inflammatory activity	[19,82]
IL-6	IL-6R, gp130	TLR4 ligands, viruses, IL-1, TNF-α	Macrophages, B cells, T cells	Pro-inflammatory cytokines; chemotaxis; induction of Th17 differentiation	[20,21,82]
IL-7	IL-7R	-	Eosinophils, B cells, T cells	Induction of Th17 activation and eosinophil development	[83,84]
IL-17A	IL-17RA, IL-17RE	IL-1β, IL-6, TGF-β	T cells, keratinocytes	Induction of Th17 activation	[20-22]
IL-18	IL-18Rα, IL-18Rβ	TLR4 ligands, staphylococcal enterotoxin, viruses, allergens *e.g.* house dust mites, IFN-γ, proteases	Basophils, DCs, macrophages, neutrophils, B cells, NK cells, NKT cells, T cells, endothelial cells, fibroblasts, keratinocytes	Pro-inflammatory cytokines; induction of IgE production and activation of Th2 and mast cells	[19,23]

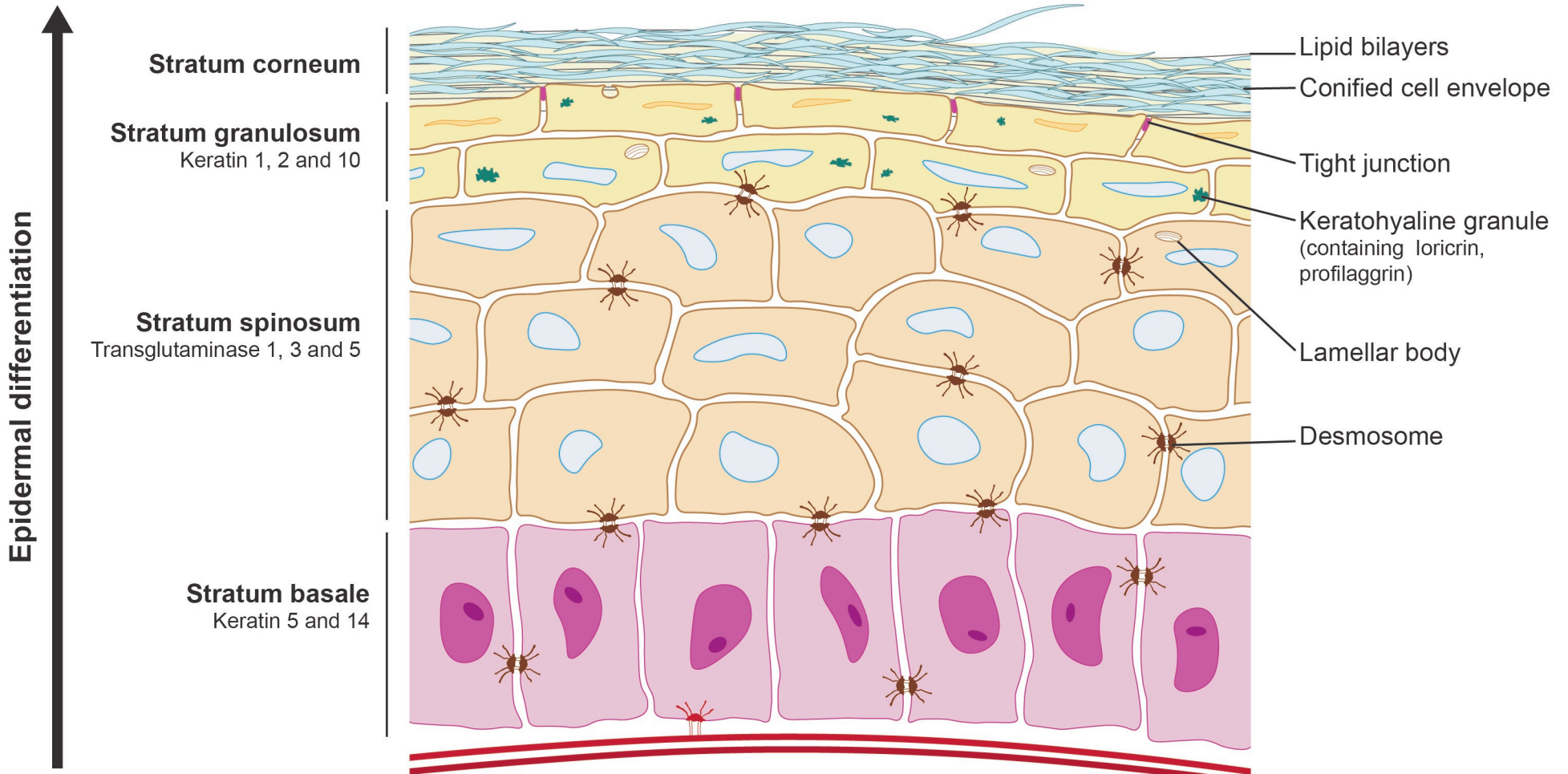
Table 1. ***Continued***

Cytokine/ chemokine	Receptor	Up-regulation	Target cell	Effect	Reference
IL-23	IL-23R, IL-12Rβ1	TLR4 ligands	DCs, LCs, NK cells, NKT cells, Th17 cells	Induction of Th17 and Th22 differentiation; promotion of epidermal thickening	[85]
IL-25 (IL-17E)	IL-17RA, IL-17RB	TLR4 ligands, ovalbumin	Basophils, eosinophils, mast cells, ILC2, NKT cells, T cells, endothelial cells	Induction of ILC2 and Th2 activation; promotion of IL-13 production and epidermal hyperplasia	[36,39]
IL-33	ST2 (IL1RL1), IL1RAcP	Allergen *e.g.* house dust mite, rhinoviruses, fungi, protease, filaggrin deficiency	Basophils, DCs, eosinophils, ILC2s, macrophages, mast cells, B cells, NK cells, T cells	Induction of Th2 immune response; activation of ILC2	[21,26,40]
GM-CSF	CD116 (GM-CSFRα)	TLR2 ligands, PMA, histamine, IL-1, TNF-α, skin barrier disruption, ultraviolet	Macrophages, T cells, keratinocytes	Stimulation of granulocyte and macrophage activation; induction of Th2 immune response	[23,84]
TNF-α	TNFR-1, TNFR-2	TLR4 ligands, mechanical injury, ultraviolet	Macrophages, NK cells, T cell, epithelial cells, fibroblasts	Proinflammatory cytokines; induction of chemoattractant and adhesion molecules ICAM-1 and CXCL-8	[23,86]

Table 1. ***Continued***

Cytokine/ chemokine	Receptor	Up-regulation	Target cell	Effect	Reference
TSLP	TSLP-receptor (IL-7Rα)	TLR1/2, TLR3, TLR4 ligands, skin barrier disruption, proteases	Basophils, DCs, eosinophils, mast cells, B cells, NKT cells, T cells	Induction of Th2 differentiation and pruritus; promotion of dendritic cell maturation	[23,26,29]

CCL, CC chemokine ligand; CTACK, cutaneous T-cell-attracting chemokine; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; gp, glycoprotein; ICAM, intercellular adhesion molecule; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IL-1RA, IL-1 receptor antagonist; IL-1RAcP, IL-1 receptor accessory protein; ILC, innate lymphoid cell; LC, Langerhans cell; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; NK cell, natural killer cell; PARC, pulmonary and activation-regulated chemokine; PMA, phorbol myristate acetate; RANTES, regulated on activation, normal T cell expressed and secreted; ST2, suppression of tumorigenicity 2; TARC, thymus and activation-regulated chemokine; TGF, transforming growth factor; Th, T helper; TLR, Toll-like receptor; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin


Epidermal differentiation
Stratum corneum
Stratum granulosum
Keratin 1, 2 and 10
Stratum spinosum
Transglutaminase 1, 3 and 5
Stratum basale
Keratin 5 and 14
Lipid bilayers
Conified cell envelope
Tight junction
Keratohyaline granule
(containing loricrin,
profilaggrin)
Lamellar body
Desmosome

Microbiota *e.g.*
S. epidermidis

Allergens/irritants

Scratch

Altered skin barrier

Pathogenic bacteria
e.g. S. aureus

Tight junction

Acanthosis

Keratinocyte

Keratinocyte

LC

DC

Proinflammatory cytokines
e.g. IL-1β, IL-6, IL-18, IL-23, GM-CSF, TNF-α

Chemokines
e.g. eotaxin/CCL11, eotaxin-3/CCL26, MCP-4/CCL13, RANTES/CCL5, TARC/CCL17

Th2 promoting cytokines
e.g. TSLP, IL-25, IL-33

HDPs
e.g. LL-37, hBDs, S100A7, RNase 7

Proinflammatory cytokines

IL-22

Th22

Histamine

IgE

Mast cell

Basophil

Eosinophil

Th17

Th17

IL-17

DC

T naive

Basophil

Eosinophil

ILC2

Th2

Th2

Th2

Th2

Th2

IL-4
IL-13
IL-31

Histamine

Eicosanoids

IL-31

IgE

Mast cell

Sensory nerve

Itch