Atopic dermatitis (AD) is a common chronic skin disease with multiple pathogenic factors including skin barrier dysfunction, immune abnormalities, and pruritus. This review summarizes components involved in these pathogenic factors. (1) Skin barrier dysfunction in AD could be due to the down-regulation of epidermal barrier components such as filaggrin, acylceramides, cornified envelope precursors, and claudin-1. (2) T helper type 2 (Th2) cell-derived cytokines, such as interleukin (IL)-4 and IL-13, and keratinocyte-derived cytokines, such as thymic stromal lymphopoietin (TSLP) and IL-33, contribute to Th2-mediated skin inflammation in AD. (3) IL-31, TSLP, IL-4, and IL-13 are able to directly activate primary sensory neurons to induce pruritus in AD, and increased susceptibility to itch (so-called allokines) is partly due to epidermal hyperinnervation. Importantly, the three key factors (skin barrier dysfunction, immune abnormalities, and pruritis) interact with each other, creating a positive feedback loop that leads to the induction and maintenance of AD. Therefore, a better understanding of not only each pathogenic factor but also their interactions is important to elucidate the complex pathophysiological mechanisms of AD, which will then lead to the development of new therapeutic strategies and drugs for the treatment of this common skin disease.

Key words  atopic dermatitis; skin barrier dysfunction; immune abnormality; pruritus

1. INTRODUCTION

Atopic dermatitis (AD) is a common chronic skin disease that usually starts in infancy, disappears or subsides with age, but sometimes continues into adulthood with relapsing course. Abnormalities in the immune system have long been recognized as contributing to the development of AD.\(^1\) On the other hand, recent accumulating evidence has highlighted a primary pathogenic role of skin barrier dysfunction in AD.\(^2,3\) Itch (or pruritus) is a hallmark of this disease and the main factor that impairs a patient’s QOL.\(^4\) Recent studies have demonstrated positive interactive effects among the three key factors: skin barrier dysfunction, immune abnormalities and pruritus. Therefore, at present, AD is assumed to result from the complex interactions of this trinity.\(^5\) This review summarizes the current understanding of the pathophysiological mechanisms of AD focusing on these three key factors and their complex interactions (Fig. 1).

2. PATHOGENIC FACTORS

2.1. Skin Barrier Dysfunction The stratum corneum (SC), the outermost layer of the epidermis, is the most important structure for skin barrier function; it is formed during epidermal differentiation. SC consists of terminally differentiated keratinocytes (called corneocytes) and intercellular lipids, which together provide a physical and hydrophobic barrier. Tight junction (TJ) proteins seal adjacent keratinocytes in the stratum granulosum, located beneath the SC, and these function as a second barrier. It has been well recognized that skin barrier function is impaired not only in lesional but also in non-lesional (i.e., clinically normal) skin of patients with AD, which was often considered secondary to skin inflammation. However, recent emerging evidence suggests that the skin barrier dysfunction in AD is actually the primary event in disease development. In this section, components involved in skin barrier dysfunction in AD are reviewed.

2.1.1. Filaggrin (FLG) FLG is a highly abundant protein expressed in differentiated epidermis. It functions to aggregate keratin filaments leading to keratinocyte compaction and SC formation. However, until just more than a decade ago, not much attention had been paid to the association of FLG with AD. Palmer et al. first reported in 2006 that a common loss of function mutation of the gene encoding FLG was highly associated with AD.\(^6\) Since then, the role of this FLG deficiency in leading to the development of AD (i.e., whether via skin barrier dysfunction) has been extensively studied. Several in vitro studies have shown that FLG deficiency indeed negatively affects epidermal permeability barrier function by perturbing epidermal differentiation or lipid composition and organization,\(^7-9\) whereas a conflicting result has also been reported.\(^10\) In vivo studies using FLG deficient mice have shown that the percutaneous penetration of hapten and protein antigens are enhanced on a
C57BL/6 background,\textsuperscript{11} and eczematous dermatitis with skin barrier impairment spontaneously develops on a proallergic BALB/c background.\textsuperscript{12} Furthermore, FLG metabolites, such as urocanic acid and pyrrolidone carboxylic acid, are shown to contribute to moisturization and maintenance of acidic pH of the SC, both of which may be crucial to epidermal barrier homeostasis by regulating the activity of multiple enzymes that control desquamation, lipid synthesis and inflammation in AD.\textsuperscript{5,13,14} Therefore, FLG deficiency could lead to impaired skin barrier function through multiple pathways, which would then drive the development of AD.

2.1.2. Acylceramides (acylCer)

Ceramides (Cer) are the dominant SC lipids. Cer not only form the extracellular lamellar membrane bilayer, but also bind covalently to highly cross-linked insoluble proteins surrounding corneocytes (called the cornified envelope, or CE). The latter forms a lipid monolayer, called the cornified lipid envelope (CLE), which is considered to act as a scaffold for organization of the extracellular lamellae. Although there exist numerous Cer species in the SC, omega-O-acylCer (or acylCer), which are unique to the epidermis, are thought to be the most critical for SC barrier function.\textsuperscript{15} Some studies have shown that the amounts of intercellular acylCer are significantly decreased in AD.\textsuperscript{16–19} Covalently bound Cer, most of which are derived from acylCer, are also reduced in both the non-lesional and lesional skin of AD patients.\textsuperscript{20,21} Furthermore, a functional deficit in acylCer may also contribute to skin barrier dysfunction in AD. Generally, acylCer contain linoleic acid, an essential fatty acid that is ester-linked to the omega-hydroxy group. Oxygenation of the linoleate by l2R-lipoxygenase and epidermal lipoxygenase-3 is a crucial step in forming CLE.\textsuperscript{22} Essential fatty acid deficiency is well known to cause severe skin barrier dysfunction, explained by the replacement of the linoleate by oleate and other non-essential fatty acids in acylCer.\textsuperscript{23} Importantly, Yamamoto \textit{et al.} found increased levels of esterified oleate (instead of linoleate) in the acylCer in the SC of AD patients.\textsuperscript{17} Abnormal Cer synthesis and metabolism, as well as essential fatty acid deficiency, have been suggested to be associated with acylCer deficits in AD.\textsuperscript{21,24–26}

2.1.3. Epidermal Differentiation Complex (EDC)

Many genes encoding proteins involved in epidermal differentiation cluster on human 1q21 (mouse 3q), known as the “EDC.” The EDC contains three families of gene encoding: (1) “CE precursors” such as loricrin, (2) “S100 proteins” such as S100A8, and (3) “S100 fused type proteins” such as FLG. Like FLG, the CE precursor proteins are also structural proteins forming the SC barrier, whereas the S100 proteins act as an antimicrobial peptide or a chemoattractant which accompanies abnormal epidermal differentiation and barrier impairment.\textsuperscript{27,28} A genome-wide linkage analysis has identified a genetic linkage of AD to chromosome 1q21.\textsuperscript{29} Furthermore, a microarray analysis of AD skin lesions has revealed the altered expression of EDC genes, such as the upregulation of S100A8 and S100A7 and the downregulation of loricrin and FLG.\textsuperscript{30} Although the EDC is also known as a susceptibility locus for psoriasis, another common inflammatory skin disorder with defective skin barrier, the expression patterns of EDC genes in AD and psoriasis are partly distinct; a comparison of gene expression profiles between psoriasis and AD skin lesions has shown that the genes encoding the CE precursor proteins are broadly defective in AD.\textsuperscript{31} Interestingly, this broad terminal differentiation abnormality was observed even in the non-lesional skin of AD patients.\textsuperscript{32} Mutations in \textit{FLG} (one of the EDC genes) are the most common risk factor for AD\textsuperscript{33}; however, a genetic study on children with severe AD suggests that the \textit{FLG} mutations are not solely responsible for the significant genetic linkage of AD to the EDC.\textsuperscript{34} Thus, the changes in other EDC genes may also contribute to AD development, possibly through multiple skin barrier defects.

2.1.4. Claudin-1

Claudin-1 is a TJ protein required for epidermal barrier integrity. It has been shown that mRNA and protein expression levels of claudin-1 are reduced in the skin of patients with AD, and polymorphisms in \textit{CLDNI} (encoding claudin-1) are associated with AD susceptibility.\textsuperscript{35} Furthermore, a recent
Several antigen-presenting cells in the skin are thought to play distinct roles in the induction of T-cell development. In the epidermis of AD, two types of dendritic cells (DCs) are increased: Langerhans cells (LCs) and inflammatory dendritic epidermal cells (IDCs), both of which express the high-affinity IgE receptor FceRI and are considered to contribute to sensitization against high molecular-weight protein antigens. On protein antigen capture, IgE-bearing LCs efficiently prime naïve T cells to differentiate into Th2 cells, whereas IDCs induce Th1 polarization by producing IL-12 and IL-18.43,44 A recent study suggests that LCs, but not IDCs, extend dendrites through TJls, which are likely to actively capture antigens from outside the TJ barrier.44 On the other hand, upon exposure to low molecular-weight happenings that easily penetrate into the dermis, dermal DCs are activated, leading to Th1 polarization.5

2.2.2. KC Innate Immune Responses
KC-mediated innate immune responses protect the host from microbial pathogens, but also drive Th2-mediated allergic diseases, including AD. KCs express pattern recognition receptors and are able to produce various chemokines and innate cytokines such as thymic stromal lymphopoietin (TSLP) and IL-33. KCs from AD patients show the abnormal production of chemokines, cytokines, and antimicrobial peptides (AMPs), suggesting some intrinsic and/or extrinsic (acquired) defects in the KCs of AD. The IL-7-like cytokine TSLP is expressed mainly by barrier epithelial cells, and activates several cell types, particularly myeloid DCs, through the TSLP receptor (TSLPR). Soumelis et al. have demonstrated that (1) TSLP activates DCs to produce Th2-attracting chemokines, (2) TSLP-activated DCs prime naïve T cells to differentiate into Th2 cells, and (3) TSLP is highly expressed in the epidermis of lesions of human AD skin, which is associated with LC migration and activation.45 In mice, KC-targeted transgenic expression of TSLP causes AD-like skin lesions accompanied by an increased infiltration of Th2 cells and elevated levels of serum IgE.46 Several studies have also shown that TSLP-TSLPR signaling on LC is critical for epicutaneous sensitization with protein antigens, as well as the development of AD-like skin inflammation in mice.5,48 These findings implicate KC-derived TSLP and TSLPR-expressing LC as key players in the pathogenesis of AD.

IL-33, a novel cytokine of the IL-1 family, is produced primarily by cells of barrier tissues (e.g., skin, gut, and lung) and mediates its biological effect via the receptor ST2. Increased expression of IL-33 and ST2 mRNA was observed in human AD skin after exposure to house dust mites or staphylococcal enterotoxin B.49 Imai et al. have demonstrated that (1) KC-specific overexpression of IL-33 in mice causes spontaneous itchy dermatitis resembling the features of AD, and (2) type 2 innate lymphoid cells (ILC2s), which produce large amounts of IL-5 and IL-13, are increased in the lesional skin and regional lymph nodes.50 Another study has further demonstrated that (1) IL-33 and ILC2s expressing ST2 are enriched in the lesional skin of AD patients, (2) IL-33-stimulated human skin ILC2s produce large amounts of type 2 cytokines, and (3) the house dust mite allergen challenge induces ILC2 infiltration into human and mouse skin.51 These findings suggest that KC-derived IL-33 induces ILC2s in the skin and lymph nodes, and stimulates the production of type 2 cytokines from those

Distinct cytokine expression patterns have been identified in the acute and chronic phases of AD skin lesions. Interleukin (IL)-4, IL-5, and IL-13 predominate in the acute lesions, whereas in the chronic lesions there is an increase of interferon (IFN)-γ, IL-12, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF).45 Thus, human AD skin is characterized roughly by Th2-cell activation in the acute phase, but by the activation of Th1 cells and/or Th0 cells (cells that share some activities of both Th1 and Th2) in the chronic phase. Animal studies using an AD model induced by the topical application of ovalbumin have shown that both Th2 and Th1 cytokines play important roles in skin inflammatory responses.47 Furthermore, it has been demonstrated that transgenic expression of either IL-4 or IL-13 in the skin induces AD-resembling phenotype in mice,38,39 and that Th1-derived IFN-γ induces apoptosis of KCs through the cell death receptor Fas,40 which may perpetuate AD. Together, these findings indicate the importance of Th2 as well as Th1 responses in the induction and maintenance of skin inflammation in AD.

On the other hand, in some hapten-induced mouse models of skin inflammation, although a single challenge provokes Th1-type contact hypersensitivity in sensitized mice, repeated elicitation results in AD-like Th2-mediated skin inflammation.41 This differential involvement of Th2 and Th1 responses in the disease stage between human and mouse models may reflect the complexity and heterogeneity of AD. Since human AD often follows a chronically relapsing course, Th2 and Th1 responses may indeed coexist in AD skin lesions. Alternatively, some Th1-dominant immune status may reflect skin pathology in a subgroup of AD. AD can be categorized into the major extrinsic (or IgE-associated) and the minor intrinsic (or non-IgE-associated) types. The latter type is immunologically characterized by the dominant Th1 response.42 The natural history of AD implies that AD manifests in the non-IgE-associated form, then shifts to an IgE-associated one.43

Recent studies have demonstrated that high-affinity IgE receptor (FcεRI) and are considered to contribute to sensitization against high molecular-weight protein antigens. On protein antigen capture, IgE-bearing LCs efficiently prime naïve T cells to differentiate into Th2 cells, whereas IDCs induce Th1 polarization by producing IL-12 and IL-18. A recent study suggests that LCs, but not IDCs, extend dendrites through TJs, which are likely to actively capture antigens from outside the TJ barrier. On the other hand, upon exposure to low molecular-weight happenings that easily penetrate into the dermis, dermal DCs are activated, leading to Th1 polarization.5

2.2. Immune Abnormalities
AD has been generally considered a T helper type 2 (Th2)-mediated allergic disease because most patients with AD have increased numbers of circulating eosinophils and elevated serum immunoglobulin E (IgE), similar to allergic asthma and rhinitis. On the other hand, previous immunopathological studies of acute and chronic skin lesions of AD patients have revealed a biphasic response in the chronic phase, but by the activation of Th1 cells and/or Th0 cells. Several studies have also shown that TSLP-TSLPR signaling on LC is critical for epicutaneous sensitization with protein antigens, as well as the development of AD-like skin inflammation in mice. These findings implicate KC-derived TSLP and TSLPR-expressing LC as key players in the pathogenesis of AD.

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cells to induce AD-like skin inflammation.

A series of studies have shown that in response to proinflammatory cytokines, KCs from patients with AD produce higher concentrations of several proinflammatory chemokines and cytokines, such as GM-CSF, IL-1α, tumor necrosis factor-α, and RANTES, compared with those from nonatopic control subjects.52,53 This enhanced KC response could promote inflammatory cell recruitment and maintain the inflammatory immune responses of AD skin.

AMPS, such as LL-37 and human beta defensin-3, are highly expressed by KCs during infection, inflammation, and wounds, which form an innate chemical barrier; however, the expression of AMPS is down-regulated by Th2 cytokines in AD.54 Thus, the deficiency of AMPS in AD skin may lead to a higher propensity toward Staphylococcus aureus infection, which is known to contribute to the exacerbation of AD.

2.3. Pruritus Most AD patients show increased itch sensitivity to minimal and negligible stimuli (this phenomenon is called alloknesis).55 Clinical studies have shown that emotional stress, sleep, and alcohol intake often trigger or enhance pruritus in patients with AD.56,57 As these triggering factors seem to primarily affect brain function, unique brain mechanisms of itch may be involved in AD. However, the pathophysiology of itch in AD is still not fully understood. Non-sedative antihistamines are less effective in ameliorating itch in AD,58 suggesting an involvement of non-histaminergic mechanisms(s). This section focuses on itch mediators, alloknesis, and brain functions involved in AD itch.

2.3.1. Itch Mediators

IL-31 is a newly identified cytokine produced by Th2 cells, and acts on the IL-31 receptor A (IL-31RA) expressed in KCs and dorsal root ganglia (DRG) neurons. IL-31 is increased in the lesional skin of AD patients.59 Skin-specific overexpression of IL-31 induces AD-like pruritic dermatitis in mice.60 An intradermal injection of IL-31 evokes acute scratching in normal mice.61 Cevikbas et al. have further demonstrated that IL-31 acts directly on a subpopulation of IL-31RA+/transient receptor potential (TRP)V1+/TRPA1+ DRG neurons to produce itch signals in mice.62 On the other hand, IL-31 does not induce immediate itch responses in humans, although about half of the subjects show late itch responses to an IL-31 challenge.63 This suggests an involvement of indirect mechanisms through other cells, such as KCs, in humans. A recent clinical study has shown that the anti-IL-31RA antibody nemilizumab significantly improves pruritus in AD patients,64 indicating the importance of IL-31 in pruritus in human AD.

A recent study has revealed that TSLP functions as a pruritogen. Wilson et al. have demonstrated that TSLP expression is increased in AD skin.65 The authors have also shown that KC production of TSLP is mediated via protease-activated receptor 2 (PAR2).66 In this regard, there is a correlation between PAR2 activity and TSLP expression in the skin of patients with Netherton syndrome with AD.67

A very recent study has identified a direct role of the canonical Th2 cytokines IL-4 and IL-13 in itch. Oetjen et al. have reported that (1) mouse and human DRG cells express both IL-4 receptor α (IL-4Rα) and IL-13 receptor α1, and are directly activated by both cytokines; (2) interestingly, IL-4 enhances neuronal responsiveness to other pruritogens; and (3) the sensory neuron-specific deletion of IL-4Rα or Janus kinase 1 (JAK1) suppresses scratching and AD-like inflammation in mice.68 These findings are consistent with a clinical study showing that a novel monoclonal antibody targeting the actions of IL-4 and IL-13 (dupilumab) significantly reduces the severity of pruritus in AD patients.69

2.3.2. Alloknesis

Alloknesis seems to be due to increased epidermal innervation, a decreased itch threshold, or both. Sprouting of nerve fibers into the epidermis is found in AD.68,69 The epidermal nerve sprouting is known to be regulated by the balance between nerve elongation factors (e.g., nerve growth factor (NGF), amphiregulin) and nerve repulsion factors (e.g., semaphoring 3A).70 Recent studies have revealed that artemin and IL-31 also contribute to nerve sprouting to produce itch.71,72 Furthermore, NGF sensitizes primary sensory neurons that respond to cowhage,73 which induces a non-histaminergic itch.

2.3.3. Brain Functions

Brain functional imaging studies have shown distinct brain activity induced by itch stimuli in patients with AD.74 Visual and auditory stimulation are known to induce itch and scratching (so-called contagious itch). Contagious itch is profound in AD patients, which correlates with activity in the fronto-striatal circuit associated with the desire to scratch.75 The molecular basis of brain mechanisms of itch in AD remains largely unclear, because there are few animal studies; however, we have shown that ethanol and barbiturates enhance scratching behavior in AD model mice through actions in the brain,76,77 and that allopregnanolone, an endogenous neurosteroid produced in the brain, also induces scratching in AD mice.78 As ethanol, barbiturates and allopregnanolone exert a hypnotic effect, the unique scratching phenomenon may mimic unconscious nocturnal pruritus frequently observed in AD patients.79

3. INTERACTIONS OF THESE PATHOGENIC FACTORS

Accumulating evidence suggests that the three pathogenic factors interact with each other, creating a positive feedback loop that contributes to the induction and maintenance of AD. In this section, their interactions are separately discussed.

3.1. Between Barrier and Immunology

Skin barrier dysfunction allows the penetration of allergens into the skin, leading to immune activation (especially Th2 responses) and skin inflammation. Experimentally, acute skin barrier disruption induces the expression of Th2 and eosinophil chemokines by epidermal cells,80,81 and promotes the activation of LCs to induce Th2 immune responses.82 As described, skin pH is partly increased by reduced FLG metabolites. The increase in skin pH directly modulates protease activity, leading to Th2-mediated inflammation via the PAR2-TSLP pathway.83

Conversely, IL-4, IL-13, and IL-33 down-regulate the expression of FLG.84,85 This could explain the fact that FLG expression is decreased, even in AD patients without FLG mutations. Other epidermal barrier components (CE, Cer, and TJs proteins) are also negatively regulated by these cytokines.86

3.2. Between Barrier and Pruritus

Skin barrier dysfunction also permits the entry of pruritogens, thereby easily inducing itch. In mice, acetone-induced skin barrier perturbation causes epidermal nerve sprouting with increased NGF
and amphiregulin levels, and tape stripping of the SC up-regulates the skin expression of TSLP. As mentioned, both nerve sprouting and TSLP induce itch. Additionally, scratching directly damages the skin barrier. Furthermore, the pruritogenic cytokine IL-31 interferes with epidermal differentiation and FLG expression, whereas it promotes epidermal proliferation and thickening, which together could lead to impaired skin barrier function.

3.3. Between Immunology and Pruritus

As described, activated Th2 cells produce several pruritogenic cytokines (i.e., IL-4, IL-13, and IL-31). Furthermore, in the chronic stage of AD, IFN-γ-induced KC apoptosis may result in the release of some pruritogens such as TSLP. Conversely, scratching exacerbates skin inflammatory immune responses. Mechanical injury to the KCs triggers pro-inflammatory cascades that contribute to the infiltration of immune cells into the skin. The “butterfly sign” (sparing of the non-reachable area of the back) is often observed in AD patients, suggesting the importance of scratching itself in the development of skin inflammation.

4. CONCLUSION

This review summarizes the mechanisms of “skin barrier dysfunction,” “immune abnormalities,” and “pruritus” as the key pathogenic factors of AD. As these factors are linked to each other, the therapeutic approach should be comprehensive. However, current therapies for AD (i.e., corticosteroids and calcineurin inhibitors) target the immune system, whereas emollients are used as an adjuvant to improve skin barrier function. There are few drugs available to treat itch in AD. Although several new medications targeting a more specific molecule (e.g., dupilumab) have been approved for the treatment of AD, and some are undergoing clinical evaluation, further studies are needed to focus on strategies to restore skin barrier function and control itch. A better understanding of the molecular basis of AD would provide important implications for the development of new therapeutic drugs for AD.

Conflict of Interest The author declares no conflict of interest.

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