# Epithelial barrier repair and prevention of allergy

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**Allergic diseases have in common a dysfunctional epithelial barrier, which allows the penetration of allergens and microbes, leading to the release of type 2 cytokines that drive allergic inflammation. The accessibility of skin, compared with lung or gastrointestinal tissue, has facilitated detailed investigations into mechanisms underlying epithelial barrier dysfunction in atopic dermatitis (AD). This Review describes the formation of the skin barrier and analyzes the link between altered skin barrier formation and the pathogenesis of AD. The keratinocyte differentiation process is under tight regulation. During epidermal differentiation, keratinocytes sequentially switch gene expression programs, resulting in terminal differentiation and the formation of a mature stratum corneum, which is essential for the skin to prevent allergen or microbial invasion. Abnormalities in keratinocyte differentiation in AD skin result in hyperproliferation of the basal layer of epidermis, inhibition of markers of terminal differentiation, and barrier lipid abnormalities, compromising skin barrier and antimicrobial function. There is also compelling evidence for epithelial dysregulation in asthma, food allergy, eosinophilic esophagitis, and allergic rhinosinusitis. This Review examines current epithelial barrier repair strategies as an approach for allergy prevention or intervention.**

Allergic diseases such as atopic dermatitis (AD), food allergy (FA), asthma, and allergic rhinitis affect more than 30% of the population (1–3). These diseases have in common a dysfunctional epithelial barrier, resulting in the penetration of allergens and microbes, accompanied by the release of epithelial-derived cytokines (e.g., thymic stromal lymphopoietin [TSLP], IL-25, IL-33), which drive type 2 immune responses. Although other immune pathways can modify the course of illness, cytokines including IL-4, IL-13, IL-31, TSLP, and IL-33 play a key role in allergic diseases (4–8), eliciting local tissue injury and repair (5, 9–14).

AD is the most prevalent chronic inflammatory skin disease (3, 15–19). In a subset of allergic patients, it is thought that AD-related skin epithelial dysfunction contributes to the atopic march, which starts with AD and often leads directly to FA (20–23). The link from AD to respiratory allergy is more controversial; however, atopic march progression is facilitated in patients who develop IgE to both food and inhalant allergens (24).

In this Review, we describe formation of the skin barrier, review the link between altered skin barrier formation and AD, discuss evidence for epithelial barrier dysfunction in other allergic diseases, and explore epithelial barrier intervention/repair strategies with the goal of preventing AD and the atopic march.

## The cornified envelope and keratinocyte differentiation in the skin

The skin's barrier function primarily depends on the outermost epidermal layer, stratum corneum (SC). To form SC, keratinocytes pass through a tightly regulated differentiation program and sequentially form stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG), and SC layers of the skin (Figure 1). In human skin, keratinocytes irreversibly exit the cell cycle after mitosis in the basal layer and differentiate progressively across the epidermis toward the SC. Each layer is defined by its expression of characteristic morphological and biochemical features indicating its state of differentiation (25–27). The keratins KRT5 and KRT14 are predominantly expressed by basal keratinocytes. The markers of early differentiation KRT1 and KRT10 are made in SS. The cornified envelope precursors involucrin (IVL) and transglutaminase-1 (TGM1) are also present in SS. Late differentiation markers including the cornified envelope protein loricrin (LOR) and the precursor of the keratin cross-linking protein filaggrin (FLG) are expressed in SG keratinocytes. TGM1 cross-links IVL, LOR, and other structural proteins to form the cornified envelope.

Calcium forms a steep gradient across the human epidermis, increasing from the SB (5 μM) to the outer SG (>20 μM) (27, 28). Importantly, there is cell-to-cell heterogeneity in average $Ca^{2+}$ concentrations in the epidermis (28). Formation of the $Ca^{2+}$ gradient coincides with key developmental milestones of skin barrier formation and differentiation into the SC.

Primary human keratinocyte cultures are an excellent model for studying epidermal differentiation, as they recapitulate the steps of epidermal cell differentiation (29). When cultured at low external calcium ($[Ca^{2+}]_o$) (~0.06 mM), epidermal keratinocytes proliferate rapidly and express a basal cell phenotype. Raising $[Ca^{2+}]_o$ above 1.3 mM (i.e., calcium switch) promotes cell differentiation, as indicated by adherens junction and desmosome formation, along with cell layer stratification and cornification. After calcium switch, changes in gene expression occur with a defined temporal order: first KRT1/10, followed by IVL and TGM1, and finally profilaggrin and LOR (30), reflecting the sequence of epidermal differentiation.

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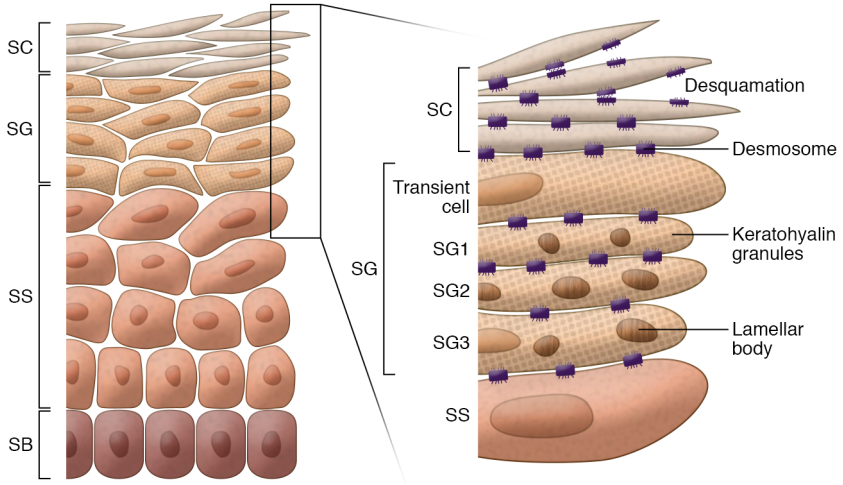


**Figure 1. Structure of human epidermis.** Human epidermis is composed of SB, SS, SG, and SC. In SC, corneocytes (flattened and denucleated keratinocytes) and intercellular lipids released from lamellar bodies form the "brick and mortar" structures. The cornified envelope, a highly cross-linked layer of insoluble proteins, forms under the corneocyte cell membrane, anchored by extracellular lipids. Components of the cornified envelope (keratohyalin granules, a source of filaggrin) and lamellar bodies (containing lipids, lipid-processing enzymes, corneodesmosin, proteases, and protease inhibitors) are formed in SG. The surface of the SC is shed off by degradation of corneodesmosomes via the activity of several proteases.

p63 transcriptional factor, described as the master regulator of skin development (31, 32), plays an essential role in maintaining self-renewing populations. p63 deficiency causes keratinocyte senescence (33), and p63-deficient keratinocytes display differentiation defects (34). Keratinocyte development in SS is selectively controlled by the Notch pathway (35–38). Notch activity in keratinocytes results in withdrawal from the cell cycle and induces expression of early differentiation markers (39) like KRT1/10, which are required for maintenance of epidermal integrity (40). KRT10-deficient mice demonstrate increased transepidermal water loss (TEWL), delayed barrier repair after barrier disruption, reduced acid sphingomyelinase (SMase) activity, and reduced ratio of ceramides to total lipids (41). Skin fragility in KRT10-deficient mice may be the consequence of two complementary mechanisms: a decrease of normal KRT1/10 filaments and an increase in KRT6/16, with a poor filament-forming capacity (42). KRT1-deficient mice also demonstrate increased TEWL and cell fragility. Transcriptome profiling revealed a KRT1-mediated gene expression signature resembling AD skin, with upregulation of S100A8, S100A9, and TSLP, compensatory increases in KRT5/14 filaments in basal epithelium, and increased KRT6/16 filaments in suprabasal epithelium (43). Skin-specific ablation of Notch produces severe AD (44, 45) and disrupted keratinocyte differentiation (46, 47). The absence of Notch activity allows Wnt/β-catenin signaling to persist in a tissue where it is normally repressed (36, 48), supporting hyperproliferation.

*Filaggrin*. SG keratinocytes produce filaggrin (FLG) as a large precursor, profilaggrin, the major component of keratohyalin granules. Profilaggrin comprises 10–12 FLG repeats flanked by single N- and C-terminal domains. At the SG-to-SC transition, profilaggrin's rapid dephosphorylation and cleavage generate positively charged FLG monomers. Profilaggrin's N-terminus translocates into the nucleus, where it may be involved in nuclear breakdown. FLG monomers, on the other hand, associate with keratin filaments and form the corneocyte fibrous matrix. In SC, after deimination by peptidylarginine deiminases, FLG becomes neutrally charged, enabling its disassociation from keratins and degradation into free amino acids, mediated by several proteases, including caspase-14, bleomycin hydrolase, and calpain-1. These amino acids (pyrrolidone carboxylic acid, urocanic acid) constitute natural moisturizing factor (NMF) and are essential for regulation of skin hydration (49), pH (50), photoprotection (50), and immune modulation (50–54).

*Formation of lipid lamellae in keratinocytes*. Lamellar bodies formed in the SS layers are organelles derived from the Golgi apparatus and contain phospholipids, glycosylceramides, sphingomyelin, and cholesterol. During the SG-to-SC transition, at the apical surface of SG cells, lamellar bodies secrete their contents (including various proteases, protease inhibitors, and lipids such as acylceramides) into the extracellular space between SG and lower SC. Once secreted from SG cells, acylceramides become part of the cornified envelope, forming repeated sheets of lipid lamellae that serve as an impermeable barrier in the SC (55).

Epidermal ceramides are critical for skin barrier function (56). Acylceramides (EOS, EOH, EOP, and EODS) account for about 12% of SC ceramides (57). Although normal ceramides contain two hydrophobic chains (sphingoid base and fatty acid), acylceramides have three hydrophobic chains (sphingoid base, an ω-OH ultra-long-chain fatty acid [ULCFA] with 30–36 carbon-chain length, and linoleic acid [C18:2-COOH]). Acylceramides serve as precursors to protein-bound ceramides for skin barrier formation. Several unique enzymes are involved in acylceramide synthesis (refs. 56–60 and Figure 2).

## Failure of terminal keratinocyte differentiation in AD skin

AD skin is characterized by broad defects in terminal keratinocyte differentiation (ref. 61 and Figure 3), expansion of cells in the SB layer, and concomitant reduction in cells of the SS and SG layers (62, 63). Hyperproliferating epithelium is associated with KRT6/16 overexpression (64). Consistent with a block in terminal keratinocyte differentiation, AD skin has reduced expression of skin barrier proteins including FLG, IVL, and LOR, antimicrobial peptides, and β-defensins (65–67).

Studies have linked *FLG* loss-of-function mutations to the structural abnormalities that underlie AD pathogenesis (17, 51). FLG-null mutations are the strongest known genetic risk factor for AD. Presence of FLG mutation increases AD risk 3-fold in comparison with the general population and predisposes to earlier disease onset, prolonged duration, and increased disease severity.

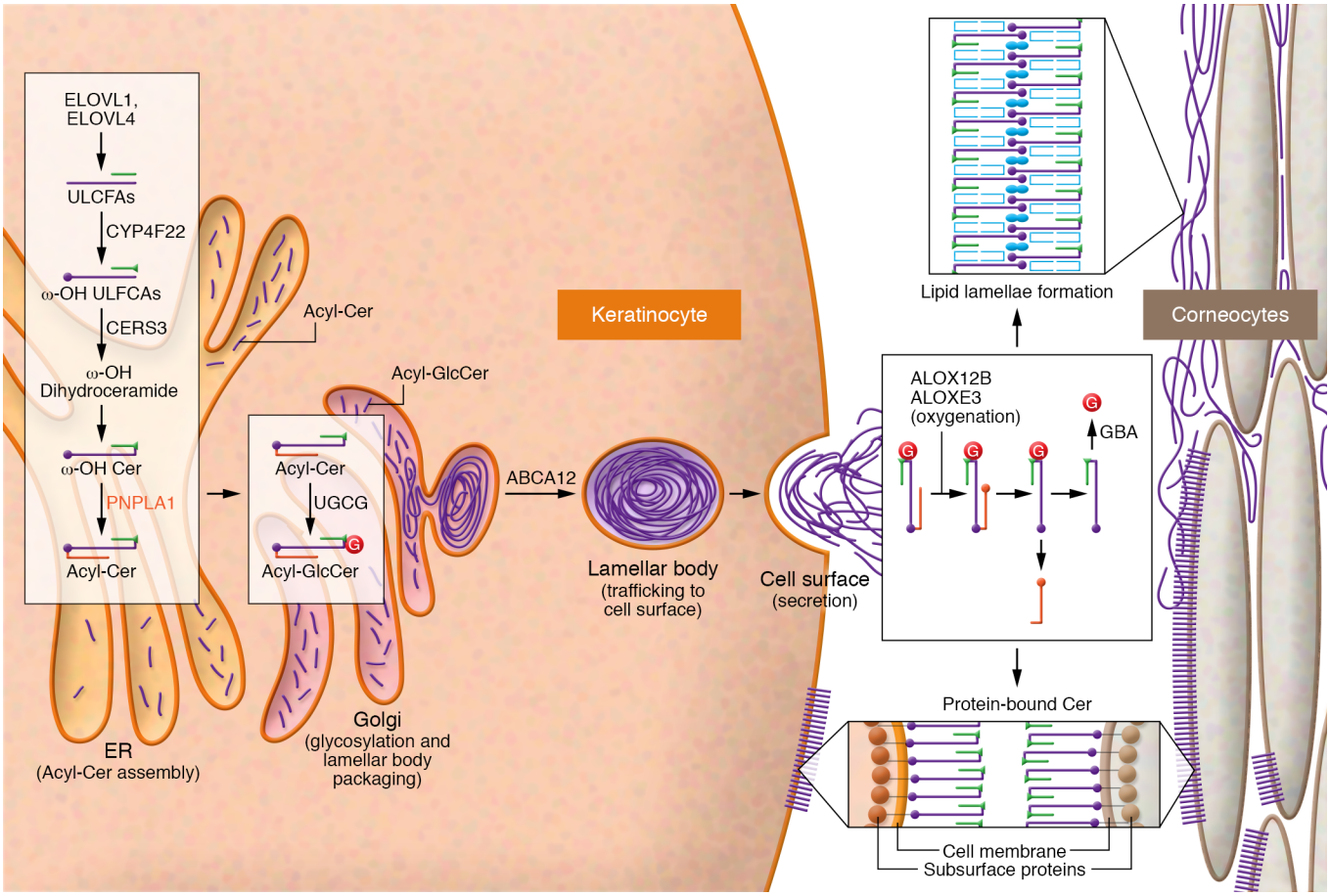


**Figure 2. Lamellar body formation and generation of acylceramides and protein-bound ceramides in human epidermis.** The enzymes and reactions responsible for creating acylceramides and protein-bound ceramides are shown. The schematics represent acylceramide production, lamellar body assembly and secretion in SG, and formation of the protein-bound ceramides in SC. Acylceramides are formed in the ER and secreted through the Golgi apparatus. ω-OH protein-bound ceramides are formed at the cell membrane. Sequential actions of the fatty acid elongases ELOVL1 and ELOVL4 generate ULCFAs of up to C26 and C28 carbon-chain-lengths, respectively. The cytochrome P450 enzyme CYP4F22 then ω-hydroxylates these ULCFAs, generating ω-OH ULCFAs. Next, the ceramide synthase CERS3 uses ω-OH ULCFAs for ω-OH ceramide synthesis. Finally, the transacylase PNPLA1 forms an ester linkage between the 18:2n-6 fatty acid taken from triglycerides and the ω-OH group of ω-OH ceramide to create an acylceramide. Each enzyme involved in acylceramide production is localized in the ER, indicating that acylceramide production takes place there. Once produced, the UDP-glucose ceramide glucosyltransferase UGCG glycosylates acylceramides in the Golgi apparatus, followed by ABCA12-mediated transport into lamellar bodies. In the course of protein-bound ceramide production, the 18:2n-6 fatty acid portions of acylceramides are subjected to peroxidation by the lipoxygenases ALOX12B and ALOXE3, followed by deglycosylation by β-glucosylceramidase (GBA). Transglutaminase then cross-links the exposed ω-OH group with cornified envelope proteins such as involucrin, envoplakin, and periplakin. Cer, ceramide; GlcCer, glucosylceramide; G, glycosyl group.

FLG mutations are the most common among Northern Europeans, with a prevalence of approximately 10%, the main mutations being R501X and 2282del4 (17). There are ethnic differences in AD-associated FLG mutations: in Asian populations, FLG P478S and C3321delA variants, uncommon in European populations, are associated with increased AD risk (68–71). In African American children, FLG2 loss-of-function mutations are associated with increased AD risk (72). Several uncommon *FLG* mutations in African American AD patients were described recently (73).

A number of additional factors play a role in regulating FLG expression in the skin (Table 1). For example, type 2 cytokines decrease *FLG* expression in AD skin, even in subjects without *FLG* mutations (52, 65). *FLG* intragenic copy number (typically 10–12 repeats) is an independent risk factor: increased copy number is protective, with each additional *FLG* repeat equating to a 12% risk reduction for AD (74). A recent paper reported that DNA methylation of the CpG site in the *FLG* gene region significantly increased AD risk (75). *FLG* is only one of approximately 45 genes within the epidermal differentiation complex (EDC) on chromosome 1q21, many of which may contribute to AD. For instance, levels of hornerin and other FLG-like proteins are decreased in AD skin (76, 77). However, FLG deficiency likely has the greatest impact in AD.

Environmental factors that adversely impact on skin barrier integrity enhance AD risk and severity. Mechanical damage, repetitive scratching, use of detergents, humidity, exposure to exogenous proteases, and air pollution also negatively impact *FLG* expression (78).

*FLG*-deficient mouse models exhibit enhanced percutaneous microbial and allergen penetration (79, 80). Similarly, examination of human skin samples determined that *FLG* mutations impair epidermal permeability barrier function (81). Knockdown of *FLG* expression in keratinocytes led to increased permeability

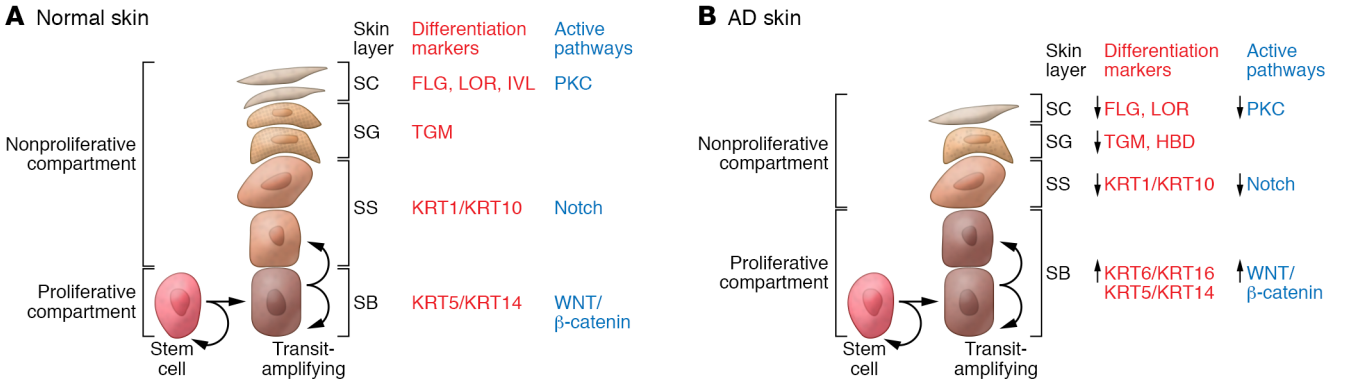


**Figure 3. Epidermal differentiation pattern in normal and AD skin.** (**A** and **B**) Epidermal differentiation pattern in normal (**A**) and AD (**B**) skin. The keratinocyte differentiation process is under tight regulation. Cells proliferate in the basal layer of the epidermis. As basal layer keratinocytes detach from the basement membrane and migrate into the first suprabasal layer in the spinous layer, they irreversibly exit the cell cycle and switch from KRT5/KRT14 to KRT1/KRT10 production. During epidermal differentiation, keratinocytes sequentially switch gene expression programs and express the granular layer differentiation markers FLG, LOR, and TGM1. The Wnt/β-catenin pathway is active in the proliferating epidermis, whereas keratinocyte differentiation in the spinous layer is under the control of the Notch pathway. Changes in extracellular $Ca^{2+}$ and lipid metabolism trigger protein kinase C (PKC) pathway activation and regulate the transcription of FLG, LOR, IVL, and TGM1. In AD skin, abnormalities in the differentiation of keratinocytes result in hyperproliferation of the basal layer, reduction of the spinous layer, and inhibition of markers of terminal differentiation, all of which compromise skin barrier and antimicrobial function.

in human keratinocyte organotypic cultures (82). Epithelial damage leads to innate immune activation, including release of proinflammatory cytokines and chemokines by keratinocytes (83), and activation of antigen presentation by skin-resident Langerhans cells and dermal DCs (84).

Electron microscopy studies illustrate that changes in epidermal *FLG* expression converge on the lamellar body secretory system to provoke skin barrier abnormality (81). Patients with *FLG* mutations demonstrate a retraction of cytosolic keratin filaments into a perinuclear shell around nuclei. This cytoskeletal abnormality appears to affect two cellular processes. First, it results in incomplete loading of cargo, including lipids, into nascent lamellar bodies, evidenced by empty microvesicles within these organelles. The resulting deposition of nonlamellar contents in the intercellular spaces then leads to focal defects in the extracellular lamellar bilayer system. Second, the cytoskeletal abnormality impairs secretion of lamellar bodies (85). *FLG*-deficient organotypic 3D skin cultures demonstrate disordered lipid lamellae (86). Another study reported decreased expression of FLG-related proteins (FLG2 and hornerin), lower expression of the FLG-degrading enzyme bleomycin hydrolase, and lower activity of another enzyme, caspase-14, in 3D cultures of keratinocytes with inhibited FLG expression (82). The mechanism for the feedback between *FLG* expression and expression/activity of FLG-processing enzymes has not been established.

Decrease in FLG expression is reflected in reduced production of FLG breakdown products, which are involved in skin acidification. This raises skin pH, thereby activating proteases (87). Activated serine skin kallikrein proteases (KLKs) interact with protease-activated type 2 receptor (PAR2), expressed by SG keratinocytes (88). PAR2 activation has been shown to inhibit lamellar body secretion in the skin (88–90) and induce the pro-Th2 cytokine TSLP (91).

Tight junctions in the SG form an additional component of the skin barrier, limiting allergen and microbe penetration, facilitating paracellular passage of soluble mediators, and regulating TEWL. Tight junctions are composed of transmembrane proteins including claudin-1 (CLDN1) that are essential for skin barrier function (92). CLDN1 levels are reduced in the skin of AD patients (93). In vitro studies have demonstrated that inhibiting CLDN1 expression enhances skin penetration (93). CLDN1 knockdown in keratinocytes enhances herpes simplex virus 1 (HSV-1) infectivity (94), particularly in patients with a history of eczema herpeticum (94).

S100A7, S100A8, and S100S9 proteins are upregulated in AD (95, 96), and their secretion amplifies skin inflammation. For instance, S100A9-activated keratinocytes selectively increase IL-33 production (95). Th2 cytokines inhibit S100A11 protein expression, which is required for the regulation of skin barrier integrity and innate immune responses (97).

Mutations in genes involved in acylceramide production (*CERS3*, *ELOVL4*, *CYP4F22*, *UGCG*, *DEGS1*, *PNPLA1*, *DGAT2*, *ALOX12B*, *ABCA12*) cause severe skin barrier defects in human patients and mouse models, and are often associated with ichthyosis symptoms (12, 56). Skin barrier lipids and proteins require coordinated assembly, and lipids may be critical to trigger profilaggrin-to-filaggrin processing (98).

**Table 1. Factors that influence filaggrin expression in the skin**

| Genetics/epigenetics | Environmental exposure | Inflammatory cytokines |
|---|---|---|
| FLG mutations | Low humidity | IL-4 |
| FLG copy number variations | Skin irritants (detergents, pollutants, hard water) | IL-13 |
| DNA methylation | Scratching | TNF-α |
| | Foods in house dust (peanut) | IL-22 |
| | | IL-25 |
| | | TSLP |
| | | IL-31 |
| | | IL-33 |

*Role of immune activation in keratinocyte dysfunction*. Decreased expression of EDC proteins (including FLG, LOR, and IVL), disruption of lipid lamellar body secretion, decreased antimicrobial peptide production (human β-defensins 2 and 3, and increased *Staphylococcus aureus* colonization can be attributable to the IL-4/IL-13 environment in AD skin (17, 64–67, 97, 99–103). Recently, clinical studies involving dupilumab, a monoclonal antibody that blocks signaling by IL-4 and IL-13, demonstrated significant clinical improvement in more than 70% of adults with moderate to severe AD (104, 105). These studies support the central role of Th2 cytokines in AD skin pathology.

IL-22, also overexpressed in AD skin, inhibits epidermal skin barrier function and suppress skin expression of FLG (106); however, in contrast to type 2 cytokines, it does not inhibit keratinocyte antimicrobial peptide production (107). Despite similar inhibitory effects on epidermal barrier function, IL-4/IL-13 and IL-22 utilize distinct receptors and transcriptional factors (IL-4Rα, IL-13Rα, common γ chain, and STAT6 versus IL-22R1, IL-10R2, and STAT3, respectively) (108, 109). Proinflammatory cytokines like TNF-α (110) and IL-33 (111) also inhibit FLG expression by keratinocytes.

*Role of microbiome in keratinocyte dysfunction in AD skin*. Abnormalities in the skin microbiome are common in AD. IL-4 and IL-13 promote *S. aureus* invasion/colonization in AD skin (67, 112–115) by inhibiting epidermal barrier function (97, 116), increasing *S. aureus* skin binding sites (e.g., fibronectin), inhibiting TLR function, and decreasing antimicrobial peptide production (67, 100, 103). Levels of staphylococcal lipoteichoic acid (LTA) from lesional AD skin correlate with the Eczema Area and Severity Index (117). Superantigen-producing *S. aureus* colonization correlates with serum IL-4 (19), and superantigens are strong activators of IL-4, IL-13, and IL-22 production in AD (118).

Longitudinal studies indicate that *S. aureus* colonization emerges during the onset of AD (119, 120). Studies emphasize a bidirectional dialogue between skin bacteria and the host organism, with microbiota activating and educating host immunity and vice versa (119, 121).

Advances in next-generation sequencing provide a more comprehensive picture of skin microbiome (122). 16s RNA sequencing and metagenomic analysis of bacterial DNA collected from AD skin documented increased *S. aureus* colonization and decreased bacterial diversity (120, 123). Specific *S. aureus* strains have been associated with AD severity (123). *S. aureus* clones identified in severe AD patients were enriched for the expression of toxin genes and genes involved in antibiotic resistance. Murine skin colonization models demonstrated *S. aureus* strain–specific differences in eliciting skin inflammation and immune signatures characteristic of AD patients. Specifically, *S. aureus* isolates from AD patients with more severe flares induced epidermal thickening and expansion of cutaneous Th2 and Th17 cells, suggesting that functional differences between staphylococcal strains may contribute to AD disease complexity (123).

Functions of normal skin microbiota include enhancement of innate immunity (124–126), limiting of pathogen invasion (126, 127), and control of Treg function (128). Birth cohort studies indicate that the presence of *Staphylococcus* species other than *S. aureus* at 2 months of life might protect infants against later dev elopment of AD (129). In contrast, early-life skin colonization with *S. aureus* may actively contribute to clinical AD onset in infancy (130). Several virulence factors, e.g., lytic toxins, enterotoxins, and proteases, produced by *S. aureus* contribute to AD pathogenesis or exacerbation through mechanisms acting on keratinocytes (cell lysis, proinflammatory cytokines production, inhibition of keratinocyte differentiation program) and immune cells (T cell activation, production of proinflammatory cytokines) (116, 131–134). *S. aureus* induces detrimental effects on keratinocytes, exploiting AD epidermal barrier defects to trigger cytokine expression (135). Activation of serine proteases is essential for *S. aureus* penetration into the skin (136).

IL-4/IL-13 overproduction provides a permissive environment for *S. aureus* growth (112, 137). Acidification of the skin by FLG breakdown products was shown to reduce expression of *S. aureus* virulence factors (138). In addition, FLG breakdown products appear to inhibit *S. aureus* expression of iron-regulated surface determinant A (138). Reduced levels of FLG breakdown products in AD skin may support *S. aureus* colonization. Th2 cytokines can also enhance the effects of staphylococcal products. For instance, compared with normal keratinocytes, AD keratinocytes have increased sensitivity to α-toxin, a cytolytic toxin produced by *S. aureus*. Differentiated keratinocytes are protected from cell death, whereas IL-4/IL-13 treatment increases sensitivity to α-toxin–induced lethality (113, 116). The combination of IL-4/IL-13 induces biochemical changes that decrease levels of acid SMase, an enzyme that cleaves the α-toxin ligand sphingomyelin (113). SMase and its enzymatic product, phosphocholine, prevent IL-4/IL-13–mediated increases in α-toxin–induced cell death (113).

*S. aureus* infection may predispose the host toward disseminated viral infections. We have demonstrated that sublytic staphylococcal α-toxin increases viral loads and promotes entry of HSV-1 and vaccinia virus (VV) into keratinocytes. VV load was significantly greater in murine skin inoculated with an α-toxin–producing *S. aureus* strain compared with murine skin inoculated with the isogenic α-toxin–deleted strain. α-Toxin's viral-enhancing effect, mediated by ADAM10, was associated with its pore-forming property (133).

We recently demonstrated an interplay between the *S. aureus* cell wall component LTA and IL-4/IL-13 in inhibiting wound healing in AD skin (139). We found that keratinocytes are highly responsive to LTA, documenting changes in expression of genes involved in regulating epidermal development, wound responses, keratinocyte proliferation, cell differentiation, and Notch signaling pathways (134). Staphylococcal LTA inhibits expression of early keratinocyte differentiation markers, including KRT1/10, and desmoglein 1 (DSG1), which are essential for skin barrier function. LTA-mediated inhibition was found to be p63 dependent (134).

*Lipid abnormalities predominate in AD skin*. Brown and colleagues stratified the analysis of AD skin transcriptome based on *FLG* gene mutations and found that patients with normal *FLG* genotype have significant changes in the expression of enzymes involved in lipid metabolism and synthesis (140). This emphasizes the importance of lipid metabolism in AD independent of *FLG* genotype. Extracellular lipids account for up to 10% of SC mass (141–143). Several skin lipids have antiinflammatory and antimicrobial properties (85, 144). Free fatty acids and sphingoid bases have documented antimicrobial activity (145–148). Several

free fatty acids serve as natural agonists for PPAR transcriptional factors (149), which are essential in regulating lipid metabolism enzymes in the skin (150).

Two independent research groups demonstrated reduced ceramide levels in AD skin in parallel with a decline in free fatty acid chain length (151–153). These changes in skin lipid composition resulted in aberrant lipid organization and positively correlated with the degree of TEWL in AD skin (85, 142). Notably, changes in ceramide levels and free fatty acid chain length distribution did not correlate with *FLG* genotype, but correlated with AD severity and levels of FLG breakdown products (151, 153). This emphasizes the importance of skin inflammation in suppressing FLG and skin barrier lipids.

Concurrent reduction in ceramide and free fatty acid chain length in the SC of AD suggests alterations in a common synthetic pathway for ceramides and free fatty acids (143, 151, 153). We hypothesized that type 2 immune response alters AD skin lipid metabolism (154). Mass spectrometric analysis of lesional SC from AD subjects and IL-13–transgenic mice revealed an increased proportion of short-chain (*N*-14:0-24:0) NS-ceramides (non-hydroxy fatty acid sphingosine ceramides), sphingomyelins, and 14:0-22:0-lysophosphatidylcholines (14:0-22:0-LPCs) with simultaneous decline in the proportion of corresponding long-chain species (*N*-26:0-32:0 sphingolipids and 24:0-30:0-LPCs) in comparison with healthy controls. An increase in short-chain LPC species was also observed in nonlesional AD skin. Similar changes were observed in IL-4/IL-13–treated $Ca^{2+}$-differentiated human keratinocytes in vitro, and all increases were blocked by siRNA-mediated silencing of STAT6, a master regulator of IL-4/IL-13 signaling. RNA sequencing analysis of AD versus healthy SC identified decreased expression of the fatty acid elongases ELOVL3 and ELOVL6, which contributed to observed changes in atopic skin lipids. Thus, our data strongly support the pathogenic role of type 2 immune activation in AD skin lipid metabolism (154). Recently, Danso et al. confirmed that alterations in the expression of key enzymes involved in SC lipid synthesis contribute to changes in the lipid composition in AD skin (155).

## Epithelial barrier dysfunction in other allergic diseases

There is compelling evidence for epithelial barrier dysfunction in other allergic diseases (10, 156). Polymorphisms in the *IL1RL1/IL18R1* locus and the *IL33* and *TSLP* genes linking epithelium-derived cytokines to type 2 inflammation in asthma have been documented (157). In addition, several polymorphisms in genes associated with epithelial homeostasis and differentiation have been identified (157–160). Increased number of goblet cells with increased mucin gene expression, increased MUC5AC protein relative to MUC5B (161–163), and reductions in ciliated cell numbers were shown in asthmatics, along with increased expression of EGFR (164) and type 2 cytokines, including IL-13, IL-5, and IL-9 (165, 166). Studies report disruption of airway epithelium in asthmatics, including loss of tight junction and reduction in adherens junction proteins. Functional studies indicate increased sensitivity of asthmatic airway epithelia to environmental stressors and oxidative stress, reducing the threshold for epithelial damage (167–169). Increased barrier permeability in asthma has been shown to promote allergic sensitization, reduce the threshold for epithelial damage, and activate type 2 responses. Changes in microbial diversity of asthmatic airways have been reported (170–173). Finally, impaired epithelial barrier repair in asthmatics leads to failure to resolve inflammatory responses (9, 10, 174).

Eosinophilic esophagitis (EoE) is considered a type 2 immune disease and often coexists with AD, asthma, and FA (13). The mucosa of the human esophagus is lined by the multilayer squamous nonkeratinized epithelium, which provides a protective barrier against environmental insults. Epithelial responses drive the majority of transcriptional changes in EoE (13, 175–177). The EoE transcriptome revealed a significant downregulation of multiple structural genes, including FLG, IVL, and the small proline-rich (SPRR) gene family (178, 179). IL-13 stimulation of esophageal epithelial cells induced disease-associated pathways, which overlapped with those observed in the esophageal mucosa of EoE patients (175, 180). Impaired barrier function is a hallmark of allergic inflammation in EoE, with inhibition of tight junction proteins (claudin-1, claudin-7, occludin), adherens junction proteins (E-cadherin), and desmosomal proteins (DSG1) compromising esophageal epithelium integrity. GWAS documented that most genes associated with increased susceptibility to EoE are expressed in the esophageal epithelium, substantiating the role of epithelial responses in EoE pathogenesis (13, 181–183). EoE-specific dysregulation is highly enriched in genes associated with protease-related activities: serine peptidase inhibitors from the SERPIN family; serine protease inhibitors, Kazal-type (SPINKs); and the protease calpain-14 (13). These proteases and their inhibitors not only regulate epithelial barrier function but are also implicated directly in inducing type 2 immune responses (13).

Altered epithelial barrier function has also been documented in allergic rhinitis, including alterations in epithelial physical barrier, mucus production, antimicrobial defense, microbiome, and immune response (184).

Epithelial-mesenchymal transition is a process during which epithelia lose many epithelial characteristics, including tight junctions, while acquiring properties of mesenchymal cells, including motility, loose cell adhesion, and depolarized cytoskeletal arrangements. Epithelial-mesenchymal transition facilitates the development of tissue fibrosis in response to injury and chronic inflammation (185). Although not described in AD, it plays an important role in asthma (186, 187) and EoE (188).

AD is often associated with sensitization to multiple foods, including peanuts, egg, milk, and tree nuts (15). A recent meta-analysis of 66 studies concluded that peanut allergy is often preceded by AD (23). Cutaneous food allergen exposure may promote sensitization (189, 190). Inflammatory cytokines released by the skin epithelium, including IL-25, IL-33, and TSLP, act on dendritic cells and other innate immune cells, leading to type 2 immune allergic response rather than tolerogenic responses (191–196). Animal studies demonstrated an essential role for TSLP in the epicutaneous induction of FA with AD-like skin lesions. Increased TSLP in the epidermis elicits the accumulation of basophils into the skin that promote type 2 cytokine responses (192). In addition, TSLP signaling on epidermal Langerhans cells may be important for IgE production during the epicutaneous sensitization to food allergens (197). In a murine model, sensitization to food allergens through an AD-like skin lesion was associated with expansion of TSLP-

**Table 2. Strategies to repair epithelial barrier function**

| Prevention | Proactive therapy (biologics) | Environmental control | Microbiome | Alternative strategies |
|---|---|---|---|---|
| Prescription emollients that mimic skin barrier lipid composition | Approved<br>Anti–IL-4R<br>Anti-IgE<br>Experimental<br>Anti–IL-13<br>Anti–IL-22<br>Anti–IL-31<br>JAK inhibitors | Dietary changes<br>Allergen avoidance<br>Avoidance of harsh detergents<br>Use of humidifiers<br>Protection from pollution | Microbial transplant<br>Commensal bacteria<br>Antibiotics | Vitamin D supplementation<br>Moisturizers<br>Occlusive agents<br>Humectants<br>FLG replacement therapies |

elicited basophils in the skin, supporting antigen-specific Th2 cytokine responses, increased antigen-specific IgE levels, and mast cell accumulation in the intestine. Notably, disruption of TSLP responses or basophil depletion reduced susceptibility to intestinal FA in this model system, whereas transferring TSLP-elicited basophils into intact skin promoted disease (193).

Importantly, several studies support the concept that children become allergic to peanuts through environmental exposure to peanut protein in household dust, especially if the infant has a disrupted skin barrier (e.g., FLG loss-of-function mutations or a history of eczema) (198, 199). Peanut protein found in the dust of the infant's home environment is biologically active and stimulates dose-dependent activation of basophils from peanut-allergic children (200).

Interestingly, a recent study (21) revealed that increased TEWL in the newborn period, even without concurrent development of AD, predicts FA occurrence at 2 years of age. Using a novel skin tape stripping (STS) technique, we found that epidermal TSLP expression occurs at 2 months of age prior to the onset of AD at 24 months of age (201). The combination of family history and increased epidermal TSLP gave an odds ratio higher than 20 for AD development.

## Strategies to improve the epithelial barrier and prevent allergy

The approaches for improving the epithelial barrier are summarized in Table 2 and detailed below.

*Prevention*. Several birth cohort studies report that the use of emollients to improve the skin barrier can prevent eczema occurrence (202, 203). In these studies, skin emollients reduced AD occurrence by approximately 50%. Transcriptomic studies found that nonlesional, normal-appearing skin of AD patients is distinct from the skin of healthy control subjects, with evidence of type 2 inflammatory responses and reduced skin barrier function in nonlesional AD (64, 140, 154, 204). It would be interesting to determine whether addition of intermittent proactive topical anti-inflammatory therapies, including low-potency topical corticosteroids (205), which are known to reduce eczema relapse down to 3 months of age, further reduces AD and FA occurrence. Application of emollients in the PEBBLES study showed a trend for decreased food sensitization at 6 and 12 months of age, but the study was not powered to measure clinical FA outcomes. Prevention of food sensitization in the PEBBLES study was more effective with early introduction (<2 weeks after birth) and frequent use (≥5 days per week) of the lipid-rich emollient (206).

*Proactive therapy*. The concomitant allergic inflammation accompanying AD can also reduce skin barrier function, and thus drive IgE responses to skin-penetrating foods. Therefore, FA prevention may require proactive skin barrier and antiinflammatory therapy to reduce type 2 immune responses to epicutaneous allergen sensitization. Alternatively, skin barrier dysfunction is well established in patients with severe eczema and type 2 immune activation. Antagonism of type 2 cytokines such as IL-4/IL-13, TSLP, or IL-33 may enhance epithelial function and reduce allergen sensitization in patients with established AD (NCT03389893 by the Atopic Dermatitis Research Network, ClinicalTrials.gov; and refs. 104, 207, 208).

*Environmental control*. Since low humidity, skin irritants (detergents), pollutants, hard water, and environmental allergens are known to induce eczema, avoidance of these environmental factors may prevent epithelial barrier dysfunction.

*Microbiome*. *S. aureus* colonization breaks down the skin barrier and predates eczema development (130, 135). Studies suggest that *S. aureus* colonization is increased on AD skin as a result of loss of commensal bacteria (127). Studies in the AD Research Network are examining whether targeted transplantation of *S. hominis* can reduce *S. aureus* colonization in AD (NCT03151148). An additional clinical study is evaluating whether topical application of Gram-negative coccobacillus *Roseomonas mucosa* can be used to treat AD (NCT03151148).

*Alternative strategies*. Vitamin D regulates FLG, LOR, IVL, and lipid lamellae formation. Maintaining normal vitamin D levels is therefore essential for development of the skin barrier (209). Moisturizers, occlusive agents, and humectants are used in AD to soften skin, create a physical barrier, and retain water (210). Experimental FLG replacement therapies currently under investigation include read-through drugs that lead to skipping of nonsense FLG mutations, drugs that regulate FLG production, FLG monomers, and FLG metabolites (12).

## Noninvasive approaches for assessing skin function

TEWL measurement is widely used to assess skin barrier function (211). During this procedure, sensors are placed in contact with the skin surface to measure water evaporating from the skin. In addition to TEWL measurements at the skin surface, TEWL measurements can be combined with STS to measure skin barrier integrity. With STS, the uppermost layers of the skin are peeled away using adhesive discs (154, 204). Skin with compromised skin barrier

exhibits greater changes in TEWL. The area under the curve for TEWL measurements over a defined number of STS reflects the overall integrity of the SC.

Our research group has pioneered novel methods to profile skin through minimally invasive and scarless STS analysis combined with transcriptomics, lipidomics, and proteomics (102, 201, 212). Using an STS protein mass spectrometry analysis, AD skin exhibits significantly lower expression of skin barrier proteins (FLG2, corneodesmosin, DSG1, DSC1, and TGM3) and enzymes (arginase-1, caspase-14, and γ-glutamyl cyclotransferase) involved in generating NMF (212, 213).

We have now extended this STS noninvasive technology to whole transcriptome sequencing together with lipidomics and proteomics. This has proven a powerful technique for agnostic examination of genomic, lipidomic, and proteomic expression profiles in nonlesional and lesional AD skin. Using these techniques, we are starting to develop new insights about AD endotypes, which include epidermal type 2 inflammation, AD patients with FA, eczema herpeticum, genetically determined deficiencies in skin cornification, and skin lipid barrier dysfunction in AD (154, 204).

## Conclusions

Clinical manifestations and skin pathology in AD are driven by impaired skin barrier and type 2–skewed immune responses.

(a) Impaired skin barrier function is caused by changes in the expression of key structural cornified barrier proteins and skin barrier lipids. *FLG* mutations are the most profound single-gene defects involved in AD (17). FLG deficiency promotes inflammation and inflammatory cell infiltration in the skin.

(b) Changes in FLG expression alter skin acidification, which, in turn, supports activation of skin proteases that alter skin barrier homeostasis by interfering with lipid lamellae assembly and support the onset of type 2 inflammatory response through TSLP and IL-33 activation (85).

(c) Type 2–skewed immune responses in AD favor epidermal barrier disruption by inhibiting the expression of FLG and other structural protein in skin (100). Th2 cytokines also inhibit production of skin barrier lipids in the skin (154). These changes are already present in nonlesional, normal-appearing AD skin (64, 154, 204), and are further aggravated in AD lesional skin.

(d) Other factors contributing to AD include *S. aureus* overgrowth and skin microbiota dysbiosis, IgE-mediated sensitization, and chronic itch. AD pathobiology is a complex interaction of epidermal barrier disruption, type 2 immune response, and imbalanced skin microbiota. Improved understanding of underlying pathology in AD will allow development of target therapies and optimize inhibition of inflammation in AD and other allergic diseases.

(e) Importantly, in a large birth cohort study, neonatal skin barrier dysfunction at 2 days of life predates the development of AD and FA later in childhood (21). This supports the notion that compromised skin barrier is critical for allergic sensitization.

(f) The interplay between genetic predisposition, microbial colonization, and type 2 inflammatory responses is important for the development of epidermal barrier abnormalities and onset of allergic responses. The key challenge now is identifying interventions to protect skin barrier function in early infancy and prevent onset of type 2 inflammatory responses and development of allergy.

(g) There is compelling evidence for epithelial dysregulation in other allergic diseases, including asthma, FA, EoE, and allergic rhinosinusitis. Through translational approaches that restore epithelial barrier homeostasis, it may be possible to prevent or modify the course of multiple allergic disorders and intervene at timeframes close to the origin of allergic diseases.

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