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Skin Barrier Defects in Atopic Dermatitis

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Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin condition with complex etiology that is dependent upon interactions between the host and the environment. Acute skin lesions exhibit the features of a Th2-driven inflammatory disorder and many patients are highly atopic. The skin barrier plays key roles in immune surveillance and homeostasis, and in preventing penetration of microbial products and allergens. Defects that compromise the structural integrity, or else the immune function of the skin barrier play a pivotal role in the pathogenesis of AD. This article provides an overview of the array of molecular building blocks that are essential to maintaining healthy skin. The basis for structural defects in the skin is discussed in relation to AD, with an emphasis on filaggrin and its genetic underpinnings. Aspects of innate immunity, including the role of anti-microbial peptides and proteases are also discussed.

Keywords

Atopic dermatitis; Th2; Inflammation; Skin barrier; Skin barrier defects; Tight junctions; Filaggrin; Defensins; Proteases; Ceramides; Epidermis

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease which is highly pruritic. Recent population-based studies in the US have estimated the prevalence of AD at over 10% in both children and adults (1, 2). While the disease often starts early in infancy, the majority of cases will resolve spontaneously; however, disease may progress into adulthood and can be debilitating. The majority of patients, including those with the most severe form of disease, are highly atopic as judged by markedly elevated levels of serum total IgE and sensitization to multiple allergens from diverse sources, including allergens and skin-colonizing microbes. The etiology of AD is multi-faceted and involves complex gene/environment interactions that conspire to undermine the first line of host defense, namely the skin.

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Conflict of Interest
Rachana Agrawal and Judith A. Woodfolk declare that they have no conflict of interest.
Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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The general view is that AD develops through Th2-driven inflammatory processes; however, T-cell infiltrates within AD skin lesions contain both Th1 and Th2 cells as well as other T cell subtypes (**Fig. 1**)(3). Priming of an array of antigen presenting cells in the skin by environmental and microbial triggers, or else by epithelial cell-derived cytokines such as TSLP and IL-33 that are secreted directly in response to these triggers, contributes to the T cell response and consequent inflammatory networks. Here, we provide an overview of the factors which compromise the structural and functional integrity of the skin barrier in AD, thereby serving to initiate and perpetuate inflammation at this site.

Barrier Defects and the Epidermis

The Epidermis

Most of the barrier defects that have been described in AD originate in the stratum corneum, the outermost layer of the epidermis. The skin provides a physical barrier against environmental insults and water loss. Both the epidermis and dermis are highly innervated and contain both resident and infiltrating immune cells. The stratified and multi-layered epidermis comprises four sub-layers which include the basal stratum germinativum, the stratum spinosum, the stratum granulosum and the top-most stratum corneum (**Fig. 2**). Keratinocytes, the predominant cell type in the epidermis, are anchored to the basement membrane by complex multi-protein structures called hemidesmosomes, and to adjacent keratinocytes by desmosomes. The skin is in a perpetual state of self-renewal. As keratinocytes migrate through the epidermis, they undergo proliferation, differentiation and finally cell-death or cornification. Within the spinous layer, keratinocytes lose their mitotic ability, change their keratin profile, and acquire lamellar granules which contain a variety of lipid precursors and enzymes. Upon arrival in the granular layer, keratinocytes become dehydrated, lose their cellular organelles and undergo further change in their protein profile in preparation for cornification. Importantly, the granular layer keratinocytes contain keratohyalin granules which carry structural proteins and their precursors such as loricrin and profilaggrin. Within the granular layer, lamellar granule contents are secreted to form the lipid layer or tight hydrophobic seal within the stratum granulosum and stratum corneum. The granular layer keratinocytes eventually undergo cell-death or cornification to form flattened corneocytes embedded in a hydrophobic lipid matrix to form the top-most impermeable cornified stratum corneum. Corneocytes are surrounded by a cornified envelope which is assembled from transglutaminase cross-linked proteins and lipids, including the major components, loricrin and involucrin. Finally, individual corneocytes are shed by desquamation, a process which maintains epidermal homeostasis by counterbalancing regeneration within the basal layer.

The Multi-Faceted Filaggrin Story

The structural protein, filaggrin, is critical to corneocyte formation in the stratum corneum. The filaggrin gene, *FLG*, encodes the 400kDa precursor polyprotein, profilaggrin, which contains 10 to12 filaggrin repeats. Monomeric filaggrin, which is produced by dephosphorylation and proteolytic cleavage of profilaggrin, aggregates and binds to keratin and other filament proteins to condense keratin bundles in the keratinocyte cytoskeleton. This process facilitates keratinocyte collapse resulting in flattened corneocytes. As such,

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filaggrin is critical to maintaining the physical strength of the stratum corneum, and minimizing entry of foreign antigens and trans-epidermal water loss (TEWL). In addition to this important attribute, filaggrin helps to maintain an acidic pH in the skin by undergoing further processing in the upper stratum corneum to release free amino acids, thereby inhibiting bacterial growth.

Filaggrin Mutations—More than thirty years ago, filaggrin was implicated in the skin disease ichthyosis vulgaris, an autosomal semidominant disorder of keratinization (4). In 1996, reduced filaggrin levels were reported in AD skin (5); however, the mechanism underlying this observation was not described until a decade later owing to the complexity of the *FLG* gene. *FLG* is a polymorphic gene comprising two small exons and a very large third exon. The third exon contains 10-12 tandem repeats of the filaggrin sequence which prevented researchers from solving its sequence using conventional PCR techniques. Finally, in 2006 the sequence of *FLG* was solved using exhaustive PCR amplification of overlapping short sequences that were then used to construct the full length sequence of exon 3 (6). This work identified two loss-of-function (null allele) mutations, R501X and 2282del4, that were associated with ichthyosis vulgaris. At the same time, these mutations, which result in truncated protein products, were also shown to be a major predisposing factor for AD (7-9). Notably, individuals harboring these mutations had disease that was early onset, more persistent, and associated with atopy (10-13). To date, 49 truncating *FLG* mutations have been identified throughouth the length of the protein (14**). The prevalence of specific *FLG* null mutations differs among different populations. Among individuals of European ancestry with AD, R501X and 2282del4 are common. For example, both mutations accounted for 80% of *FLG* mutations among an Irish cohort of patients with AD as compared with <2% among patients from Singapore (14, 15). In addition, the spectrum of *FLG* null mutations varies between European and Asian populations. For example, c. 3321delA and S1515X, which are present in Asian populations, have not been identified in European populations to date (14).

The variable number of repeats encoding filaggrin monomer within *FLG* exon 3 may also contribute to decreased levels of filaggrin in AD skin. Consistent with this view, intragenic copy number variation in *FLG* contributes to disease severity as evidenced by lower copy numbers in AD patients as compared with controls (16). In addition, methylation of *FLG* was recently shown to add to disease risk associated with null variants, thereby establishing a potential interaction between epigenetic and genetic factors in determining disease outcomes (17).

Several lines of evidence now support a role for *FLG* mutations and genetic variations in promoting perturbation of the skin barrier at the cellular level. These include an association between *FLG* genotype or deficiencies in filaggrin, and disorganized keratin filaments, abnormal bilayer architecture, impaired lipid profiles and altered acidification pathways in skin (18-20). Other studies have demonstrated effects on TEWL and hydration (21, 22). Deficiencies in filaggrin protein may extend beyond structural impairment to the immune system through effects on pro-inflammatory cytokines. Increased IL-1 expression has been reported in the stratum corneum of AD patients with *FLG* mutations as compared to those

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lacking *FLG* mutations (23). Similarly, in a mouse model, filaggrin deficiency has been implicated in induction of the Th2-promoting cytokine TSLP in keratinocytes (24).

Filaggrin By-products—In healthy skin, filaggrin is degraded by the cysteine peptidase, caspase-14, and other proteases into natural moisturizing factors (NMF)(25). These include free amino acids, urocanic acid and pyrrolidine carboxylic acid. NMFs work to balance moisture, pH and buffering capacity, thereby strengthening the stratum corneum barrier not only against environmental insults such as allergens and chemical pollutants, but also against colonization or infection by bacteria and other pathogens. Reduced NMF levels have been reported in AD patients carrying *FLG* mutations as compared with their counterparts lacking these mutations (21, 26, 27).

Filaggrin Deficiency and Antigen Co-Exposure—Not all subjects who harbor *FLG* mutations develop disease. Conversely, not all AD patients carry *FLG* mutations. Thus, despite the evidence of a role for filaggrin, other factors clearly influence the path to disease. In filaggrin-deficient or filaggrin-null mouse models, a compromised skin barrier is not sufficient to induce AD. In these mice, percutaneous co-exposure to allergens or protein antigens such as ovalbumin or topical haptens and a resultant IgE mediated immune response appears to be required (28*-31). A common feature of these models was an enhanced percutaneous immune response, suggesting enhanced antigen penetration. However, it is not clear whether this phenomenon translates to humans. For example, filaggrin knock-down alone did not affect the permeability, lipid organization or composition of the stratum corneum in an *ex vivo* human skin model (32). Moreover, in AD patients with and without *FLG* mutations who were sensitized on healthy skin by cutaneous application of 2,4-dinitrochlorobenzene (DNCB), equal penetration of the skin and similar Th2 skewed DNCB-specific T cell responses were observed between groups (33). By contrast, other work supports an interaction between *FLG* mutations and allergen exposure. Among children who were monitored for the first 8 years of life, exposure to cat in early life significantly enhanced the risk of developing eczema associated with *FLG* mutations (34). Further work is clearly warranted to understand how *FLG* mutations impact antigen-driven immune responses through the skin.

Immune Modulation of Filaggrin—Acute skin lesions in AD exhibit the hallmarks of Th2-driven inflammation including high expression of TSLP in the epithelium and the presence of Th2 cells expressing IL-4 and IL-13, and basophils (35, 36). Recent evidence supports a role for Th2-associated factors in regulating filaggrin expression. In human skin models and keratinocyte cultures, histamine and a variety of cytokines including IL-4, IL-17, IL-22, IL-25 and IL-31 have been shown to decrease filaggrin production or else repress *FLG* gene expression (37-43). Certain cytokines may modulate expression of filaggrin levels indirectly, as evidenced by cytokine-mediated downregulation of proteases involved in processing of profilaggrin (see below). Evidence of an interaction between genetic polymorphisms in IL-10 and IL-13 with the 2284del4 *FLG* mutation and enhanced risk of AD supports the view that the interplay between cytokines and filaggrin levels in the skin is complex (44).

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Filaggrin-like Proteins—Expression profiles of filaggrin and filaggrin-like proteins continue to be elucidated in healthy and diseased skin. Deficiencies in epidermal filaggrin-like proteins, filaggrin-2 and hornerin, have recently emerged in AD (45, 46). Genes encoding these proteins, along with filaggrin, map to the epidermal differentiation complex on human chromosome 1q21 which comprises a large number of genes involved in maturation of the epidermis. Similar to filaggrin, filaggrin-2 belongs to the S100 fused-type protein family and shares many molecular attributes with filaggrin. Two premature stop codon mutations in *FLG2*, designated rs12568784 and rs16833974, have recently been associated with more persistent AD (47). Recently, a single nucleotide polymorphism in the hornerin gene, *HRNR* (rs877776), was reported in German but not Irish patients with AD (48, 49).

Other Proteins in the Stratum Corneum

Corneocytes in the uppermost epithelial layer are surrounded by a cornified envelope and attached to adjacent corneocytes by corneodesmosomes. In addition to highly reduced compaction of corneocytes in AD skin, there is evidence of defects in the cornified envelope (50). The corneodesmosomal proteins, desmoglein-1, desmocolin-1 and corneodesmosin are expressed at lower levels in AD lesional skin than non-lesional skin (45). Also, the staining patterns of corneodesmosomal proteins have been shown to be abnormally diffuse (51). In addition, loricrin and involucrin are expressed at lower levels in AD skin as compared with healthy skin (50, 52, 53). In a mouse model, combined deficiencies in the scaffold proteins of the cornified envelope, involucrin, envoplakin and periplakin, resulted in hyperkeratosis, as well as decreased lipid content and reduced proteases resulting in defective filaggrin processing. Moreover, cellular infiltrates of $CD4^+$ T cells and T cells were evident (54). In human keratinocyte cultures, expression of corneodesmosome and cornified envelope proteins is decreased by Th2-associated factors including IL-4, IL-13 and histamine, suggesting that the inflammatory milieu in AD skin compromises the cornified envelope (37, 53).

The Lipid Bilayer

Hydrophobic lipids form the ‘mortar’ of the stratum corneum, and serve to strengthen and maintain hydration of the epidermal barrier. Keratinocytes in the stratum granulosum release lipid precursors and lipid processing enzymes from their lamellar granules in the uppermost layers of the epidermis. The predominant lipid precursors comprise glycosylceramides, sphingomyelin and phospholipids which are enzymatically processed to form ceramides, cholesterol and free fatty acids. In healthy skin, ceramides constitute the predominant lipids. Early evidence implicating the lipid bilayer in compromised barrier function in AD came from electron microscopy studies which revealed disturbed or incomplete lamellar body maturation and extrusion (55, 56). Since that time, several studies have convincingly demonstrated not only decreased levels of ceramides but also changes in ceramide composition or in the ceramide/cholesterol ratio in AD skin (57-59). In addition, to these quantitative changes in lipid composition, the fatty acids and ceramides found in AD skin have a shorter chain length than in normal skin, which may enhance permeability of the skin barrier (60, 61). These abnormalities have been correlated with abnormal lipid organization in the stratum corneum of AD patients as judged by electron diffraction studies (60, 62).

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Decreased ceramide levels in AD skin might arise from increased expression of lipid-processing enzymes such as sphingomyelinase and sphingomyelinase deacylase, or else from expression of novel enzymes such as glucosylceramide deacylase (63-65). Beyond these aspects, other studies have implicated the immune system in altering lipid metabolism in AD. In a human *ex vivo* skin model, IL-4 and IL-6 decreased ceramide levels while IFN-γ and TNF-α had the opposite effect (66). However, in other work, IFN-γ decreased ceramides with long chain fatty acids suggesting that it may contribute to altered ceramide profiles in AD skin (67). IFN-γ can be expressed at high levels in AD skin wherein it mediates enhanced apoptosis of keratinocytes in a Fas-dependent manner (68). Thus, this cytokine acts through multiple pathways to undermine the skin barrier.

Tight Junctions

Tight junctions are highly complex structures which form a paracellular barrier to tightly regulate passage of solutes in a highly selective fashion across the epithelium (69). The components of tight junctions include claudins, occludins, tricellulin, zonula occludens and junction adhesion molecules. Among these, the claudin (CLDN) family of proteins, which consists of more than 20 proteins, is pivotal to tight junction formation. Within the skin, the composition of tight junction complexes differs in different layers of the epidermis. Moreover, as knowledge of tight junctions has evolved, it has become clear that these complexes fulfill functions beyond their structural role. These aspects are beyond the scope of this article and are reviewed elsewhere (70).

In healthy skin, Langerhans cells are able to sample antigens that have gained access through the stratum corneum, by extending their dendrites to penetrate tight junctions between keratinocytes (71). This remarkable adaptability facilitates immune surveillance while maintaining integrity of the skin barrier. The majority of AD patients who are atopic are sensitized to dust mite. Furthermore, IgE antibodies to microbial products derived from skin-colonizing organisms can be detected in the serum of these patients, suggesting that the skin acts as a portal for enhanced antigen entry (72 73). The cysteine protease dust mite allergen, Der p 1, has the capacity to cleave occludin, and putatively claudin-1, thereby breaching tight junctions at epithelial surfaces (74). Similarly, in an *ex vivo* model of porcine skin that was infected using *Staphylococcus spp.*, disruption of tight junctions was evidenced by downregulation of a variety of proteins including occludin and zonula occludens-1, coupled with an increase in transepidermal resistance (75). Reduced levels of CLDN1 in AD skin, or else in human keratinocytes, have been inversely correlated with expression of Th2 markers and a propensity to infection with herpes simplex virus 1 (76*, 77). Together, these findings support a key role for reduced CLDN1 in immune dysregulation and susceptibility to infection in AD.

Anti-Microbial Peptides

The skin provides the first outpost of the immune system. As such, its ability to mount an innate response to invading pathogens is pivotal to the host's wellbeing. In AD patients, the prevalence of skin colonization with *Staphylococcus spp.* is high. Furthermore, a subset of patients are predisposed to develop eczema herpeticum, a disseminated infection with herpes simplex virus. These features of the disease point to critical defects in the innate response.

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Antimicrobial peptides (AMPs) which target bacteria, fungi and viruses, are integral to this response. AMPs are small cationic proteins that permeabilize microbial membranes through interactions with anionic components, resulting in microbial lysis. Specific AMPs target specific pathogens by virtue of their charge and structure which dictate the mechanism of microbial killing. In addition to their direct anti-microbial activity, AMPs also recruit inflammatory cells and stimulate expression of cytokines and chemokines. Whereas in healthy skin AMPs are produced by keratinocytes, they are produced by a variety of cell types in inflamed skin, including infiltrating neutrophils and resident mast cells (78). AMPs can be subdivided into several families, among which, β-defensins and cathelicidins are the most highly elucidated. In humans, β-defensin-1 (hBD-1) is constitutively expressed while hBD-2 and hBD-3 are induced in response to inflammatory stimuli, via toll-like receptor signaling pathways. A complete list of AMPs in human skin is described elsewhere (78).

The role of AMPs in AD skin is complex and controversial. Initial studies aimed at understanding the role of AMPs in AD questioned why AD patients are more prone to colonization by microbial pathogens than those with psoriasis. Comparison of messenger RNA or protein levels of AMPs in lesional skin from patients with AD and psoriasis revealed markedly lower expression of the cathelicidin LL-37, and hBD-2 in AD skin (79*, 80). Other studies which focused on hBD-3, and dermcidin, an AMP found in sweat glands, supported these observations (81, 82). Decreased LL-37, hBD-2 and hBD-3 have also been reported in the skin of AD patients colonized by herpes simplex virus compared with non-colonized patients (83, 84).

On the other hand, there is a body of work to support the view that AMPs are actually induced in AD skin. Higher levels of LL-37 have been reported in lesional versus non-lesional AD skin (85). Similarly, increased hBD-2, hBD3 and RNase 7 have been shown in AD skin as compared to healthy skin at the mRNA or protein level (86, 87). Such discrepancies may relate to injury at the skin biopsy site. For example, tape stripping can enhance expression of a variety of factors including psoriasin, RNase 7, hBD-2 and hBD-3 in lesional skin (88, 89). Other contributing factors might include atopic status of the patient; bacterial colonization profiles of the skin at the time of biopsy; chronicity of the lesion; the type of skin specimen obtained (partial- or full-thickness); and the methods used to identify AMPs.

While there is evidence of decreased activity of AMPs in AD skin, it is not clear whether this results from decreased production, or else decreased mobilization from keratinocytes with a consequent impairment in microbial killing (90). In addition, genetic factors may also contribute. Though various single nucleotide polymorphisms in *DEFB1*, the gene encoding hBD-1 have been associated with AD, the functional relevance of such genetic variations is not clear (91, 92). In order to better understand the mechanisms controlling levels of AMPs in the skin, investigators have explored the modulatory properties of cytokines. The Th2 cytokines, IL-4 and IL-13 can inhibit hBD-2 and hBD-3 gene expression by keratinocytes *in vitro* (81). Furthermore, antibody-mediated neutralization of IL-4, IL-10 and IL-13 enhanced expression of LL-37, hBD-2 and hBD-3 in skin explant cultures from AD patients (79*, 93, 94). Notably, inhibition of IL-4 and IL-13 also inhibited replication of vaccinia virus in keratinocyte cultures (79). This key observation suggests that Th2 cytokine expression in the

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skin contributes to the risk for developing eczema vaccinatum in AD patients following viral inoculation. Interleukin-33 is a newly described cytokine that is expressed by epithelial cells and activates a variety of cell types including Th2 cells and mast cells. Inhibition of serum-induced hBD-2 in human keratinocyte cultures has recently been described (95). Collectively, these observations highlight the complexity of expression and regulation of AMPs in AD skin.

Proteases and Their Inhibitors

Proteases and their inhibitors are central to barrier homeostasis by maintaining the desquamation process. They aid in the degradation of the corneodesmososmes which tether adjacent corneocytes, thereby facilitating detachment of the corneocytes from the stratum corneum without compromising the integrity of the skin barrier. Proteases also act to regulate levels of lipid-processing enzymes and anti-microbial peptides; and are involved in chemotaxis, cytokine expression, inflammation, tissue repair and apoptosis. Among the extensive array of proteases expressed in skin, those that have been most frequently implicated in AD are the kallikreins (KLK). These serine proteases mediate their functions through activation of G-protein coupled protease-activated receptors (PARs) expressed on a broad array of cell types. Perturbation of protease production and activation appears to lead to abnormal desquamation and breakdown of lipid-processing enzymes and AMPs. Several studies point to aberrant expression and enhanced enzymatic activity of kallikreins, in AD skin (96-98). Genetic studies have identified a 4bp-insertion in the 3'untranslated region of the *KLK7* gene which is associated with AD, but only in those patients who have lower serum IgE levels (99). However, in another study, the same insertion in *KLK7* was not linked to AD (100). KLK5 binds to PAR-2 which is expressed at high levels on primary afferent nerve fibers and keratinocytes in AD skin biopsies. This receptor mediates itch, as well as upregulation of ICAM-1, and the production of pro-inflammatory cytokines in animal systems (101, 102). Furthermore, in cultures of normal human keratinocytes, IL-4 and IL-13 can enhance the expression and activity of KLK7, suggesting that Th2 cytokines themselves contribute to kallikrein dysfunction (103). Other proteases that have been implicated in AD include the cysteine protease cathepsins. Cathepsin V levels are decreased in AD skin lesions and this is linked to increased expression of cystatin M/E which exerts anti-protease activity (104). However, in animal models, both decreased and increased expression of cathepsins leads to inflammatory responses in the skin akin to those in AD (105, 106). Caspase-14 is a cysteine peptidase that is expressed primarily in keratinocytes and plays a role in terminal differentiation. Caspase-14 was recently shown to process filaggrin monomers and degrade filaggrin, resulting in the production of NMFs (25). Deficiencies in caspase-14 in AD skin have been observed; however, how this may relate to inflammatory responses remains to be determined (107).

The importance of protease inhibitors in maintaining the skin barrier is evidenced by Netherton Syndrome, an autosomal recessive disorder resulting from a loss-of-function mutation in the *SPINK5* gene (108*). This gene encodes the protease inhibitor, lymphoepithelial Kazal-type related inhibitor (LEKTI) which, among other proteases, targets KLK5 and KLK7. Individuals affected by this syndrome develop severe AD and allergic symptoms arising from dysregulated protease activity. This results in thinning of the

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stratum corneum and markedly increased production of TSLP by epithelial cells via the KLK5/PAR2 axis (109, 110). Several genetic studies have linked single nucleotide polymorphisms in *SPINK5* to atopy and AD; however, the data is conflicting (111-114).

Conclusions and Future Directions

There is overwhelming evidence that defects in the skin barrier contribute to the pathogenesis of AD (Table 1). While the discovery of filaggrin mutations represents a major advance in our understanding of the disease, it does not tell the whole story. Barrier defects are determined by a constellation of host and environmental factors. In addition to reduced levels of filaggrin, irregularities in other structural proteins conspire to compromise the skin barrier, thereby facilitating antigen entry and the breakdown of host defenses. Deficiencies in innate immunity further undermine the skin barrier and increase the propensity for infection and aberrant adaptive immune responses. Understanding how these events unfold over time will be pivotal to identifying new molecular targets for therapy. Topical treatments which restore the skin barrier in AD are an important modality in the armory against AD. Incorporation of recombinant filaggrin monomers is one approach currently being tested in animal models (115). As we learn more about the skin barrier and its defects in AD, we can anticipate new developments in topical therapies for this debilitating disease.

Acknowledgments

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Abbreviations

AD	atopic dermatitis
AMPs	anti-microbial peptides
CLDN	claudin
DNCB	2,4-dinitrochlorobenzene
KLK	kallikrein
NMF	Natural moisturizing factors
PAR	protease-activated receptor
TEWL	trans-epidermal water loss

References

1. Shaw TE, Currie GP, Koudelka CW, Simpson EL. Eczema prevalence in the United States: data from the 2003 National Survey of Children's Health. J Invest Dermatol. 2011. 131:67–73.
2. Silverberg JI, Hanifin JM. Adult eczema prevalence and associations with asthma and other health and demographic factors: a US population-based study. J Allergy Clin Immunol. 2013; 132:1132–8. [PubMed: 24094544]
3. Novak N, Bieber T, Leung DY. Immune mechanisms leading to atopic dermatitis. J Allergy Clin Immunol. 2003; 1112:S128–39. [PubMed: 14657843]

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4. Sybert VP, Dale BA, Holbrook KA. Ichthyosis vulgaris: identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. J Invest Dermatol. 1985; 84:191–4. [PubMed: 2579164]
5. Seguchi T, Cui CY, Kusuda S, Takahashi M, Aisu K, Tezuka T. Decreased expression of filaggrin in atopic skin. Arch Dermatol Res. 1996; 288:442–6. [PubMed: 8844122]
6. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet. Mar. 2006; 38(3):337–42. [PubMed: 16444271]
7. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006; 38:441–6. [PubMed: 16550169]
8. Sandilands A, O'Regan GM, Liao H, Zhao Y, Terron-Kwiatkowski A, Watson RM, et al. Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. J Invest Dermatol. 2006; 126:1770–5. [PubMed: 16810297]
9. Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol. 2006; 118:214–9. [PubMed: 16815158]
10. Weidinger S, Rodriguez E, Stahl C, Wagenpfeil S, Klopp N, Illig T, et al. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J Invest Dermatol. 2007; 127:724–6. [PubMed: 17096018]
11. Barker JN, Palmer CN, Zhao Y, Liao H, Hull PR, Lee SP, et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol. 2007; 127:564–7. [PubMed: 16990802]
12. Henderson J, Northstone K, Lee SP, Liao H, Zhao Y, Pembrey M, et al. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. J Allergy Clin Immunol. Apr.2008 121:872–7. e9. [PubMed: 18325573]
13. Brown SJ, Sandilands A, Zhao Y, Liao H, Relton CL, Meggitt SJ, et al. Prevalent and low-frequency null mutations in the filaggrin gene are associated with early-onset and persistent atopic eczema. J Invest Dermatol. 2008; 128:1591–4. [PubMed: 18094728]
14••. Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. N Engl J Med. Oct 6. 2011; 365(14):1315–27. [PubMed: 21991953] [An excellent review article describing filaggrin mutations, including seminal work which led to their link with ichythosis vulgaris and atopic dermatitis.]
15. Chen H, Common JE, Haines RL, Balakrishnan A, Brown SJ, Goh CS, et al. Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singaporean Chinese and European populations. Br J Dermatol. 2011; 165:106–14. [PubMed: 21428977]
16. Brown SJ, Kroboth K, Sandilands A, Campbell LE, Pohler E, Kezic S, et al. Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect. J Invest Dermatol. 2012; 132:98–104. [PubMed: 22071473]
17. Ziyab AH, Karmaus W, Holloway JW, Zhang H, Ewart S, Arshad SH. DNA methylation of the filaggrin gene adds to the risk of eczema associated with loss-of-function variants. J Eur Acad Dermatol Venereol. 2013; 27:e420–3. [PubMed: 23003573]
18. Gruber R, Elias PM, Crumrine D, Lin TK, Brandner JM, Hachem JP, et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. Am J Pathol. 2011; 178:2252–63. [PubMed: 21514438]
19. Mildner M, Jin J, Eckhart L, Kezic S, Gruber F, Barresi C, et al. Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. J Invest Dermatol. 2010; 130:2286–94. [PubMed: 20445547]
20. Vavrova K, Henkes D, Struver K, Sochorova M, Skolova B, Witting MY, et al. Filaggrin Deficiency Leads to Impaired Lipid Profile and Altered Acidification Pathways in a 3D Skin Construct. J Invest Dermatol. Sep 23.2013 [Epub ahead of print].
21. Kezic S, Kemperman PM, Koster ES, de Jongh CM, Thio HB, Campbell LE, et al. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. J Invest Dermatol. 2008; 128:2117–9. [PubMed: 18305568]

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22. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H. Clinical severity correlates with impaired barrier in filaggrin-related eczema. J Invest Dermatol. 2009; 129:682–9. [PubMed: 18818676]
23. Kezic S, O'Regan GM, Lutter R, Jakasa I, Koster ES, Saunders S, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. J Allergy Clin Immunol. 2012; 129:1031–9. e1. [PubMed: 22322004]
24. Moniaga CS, Jeong SK, Egawa G, Nakajima S, Hara-Chikuma M, Jeon JE, et al. Protease activity enhances production of thymic stromal lymphopoietin and basophil accumulation in flaky tail mice. Am J Pathol. 2013; 182:841–51. [PubMed: 23333753]
25. Hoste E, Kemperman P, Devos M, Denecker G, Kezic S, Yau N, et al. Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin. J Invest Dermatol. 2011; 131:2233–41. [PubMed: 21654840]
26. Kezic S, O'Regan GM, Yau N, Sandilands A, Chen H, Campbell LE, et al. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. Allergy. 2011; 66:934–40. [PubMed: 21261659]
27. O'Regan GM, Kemperman PM, Sandilands A, Chen H, Campbell LE, Kroboth K, et al. Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. J Allergy Clin Immunol. 2010; 126:574–80. [PubMed: 20621340]
28. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet. 2009; 41:602–8. [PubMed: 19349982]
29. Kawasaki H, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, et al. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. J Allergy Clin Immunol. 2012; 129:1538–46. [PubMed: 22409988]
30. Scharschmidt TC, Man MQ, Hatano Y, Crumrine D, Gunathilake R, Sundberg JP, et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. J Allergy Clin Immunol. 2009; 124:496–506. [PubMed: 19733297]
31. Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. J Allergy Clin Immunol. 2009; 124:485–93. [PubMed: 19665780]
32. van Drongelen V, Alloul-Ramdhani M, Danso MO, Mieremet A, Mulder A, van Smeden J, et al. Knock-down of filaggrin does not affect lipid organization and composition in stratum corneum of reconstructed human skin equivalents. Exp Dermatol. 2013; 22:807–12. [PubMed: 24164439]
33. Newell L, Polak ME, Perera J, Owen C, Boyd P, Pickard C, et al. Sensitization via healthy skin programs Th2 responses in individuals with atopic dermatitis. J Invest Dermatol. 2013; 133:2372–80. [PubMed: 23528819]
34. Schuttelaar ML, Kerkhof M, Jonkman MF, Koppelman GH, Brunekreef B, de Jongste JC, et al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. Allergy. 2009; 64:1758–65. [PubMed: 19839980]
35. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol. 2002; 3:673–80. [PubMed: 12055625]
36. Ito Y, Satoh T, Takayama K, Miyagishi C, Walls AF, Yokozeki H. Basophil recruitment and activation in inflammatory skin diseases. Allergy. 2011; 66:1107–13. [PubMed: 21371044]
37. Gschwandtner M, Mildner M, Mlitz V, Gruber F, Eckhart L, Werfel T, et al. Histamine suppresses epidermal keratinocyte differentiation and impairs skin barrier function in a human skin model. Allergy. 2013; 68:37–47. [PubMed: 23157658]
38. Gutowska-Owsiak D, Schaupp AL, Salimi M, Selvakumar TA, McPherson T, Taylor S, et al. IL-17 downregulates filaggrin and affects keratinocyte expression of genes associated with cellular adhesion. Exp Dermatol. 2012; 21:104–10. [PubMed: 22229441]
39. Gutowska-Owsiak D, Schaupp AL, Salimi M, Taylor S, Ogg GS. Interleukin-22 downregulates filaggrin expression and affects expression of profilaggrin processing enzymes. Br J Dermatol. 2011; 165:492–8. [PubMed: 21564072]

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40. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol. 2009; 124(3 Suppl 2):R7–R12. [PubMed: 19720210]
41. Hvid M, Vestergaard C, Kemp K, Christensen GB, Deleuran B, Deleuran M. IL-25 in atopic dermatitis: a possible link between inflammation and skin barrier dysfunction? J Invest Dermatol. 2011; 131:150–7. [PubMed: 20861853]
42. Kim BE, Bin L, Ye YM, Ramamoorthy P, Leung DY. IL-25 enhances HSV-1 replication by inhibiting filaggrin expression, and acts synergistically with Th2 cytokines to enhance HSV-1 replication. J Invest Dermatol. 2013; 133:2678–85. [PubMed: 23657503]
43. Cornelissen C, Marquardt Y, Czaja K, Wenzel J, Frank J, Luscher-Firzlaff J, et al. IL-31 regulates differentiation and filaggrin expression in human organotypic skin models. J Allergy Clin Immunol. 2012; 129:426–33. [PubMed: 22177328]
44. Lesiak A, Kuna P, Zakrzewski M, van Geel M, Bladergroen RS, Przybylowska K, et al. Combined occurrence of filaggrin mutations and IL-10 or IL-13 polymorphisms predisposes to atopic dermatitis. Exp Dermatol. 2011; 20:491–5. [PubMed: 21426411]
45. Broccardo CJ, Mahaffey S, Schwarz J, Wruck L, David G, Schlievert PM, et al. Comparative proteomic profiling of patients with atopic dermatitis based on history of eczema herpeticum infection and Staphylococcus aureus colonization. J Allergy Clin Immunol. 2011; 127:186–93. [PubMed: 21211653]
46. Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Mechin MC, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. J Allergy Clin Immunol. 2013; 131:1094–102. [PubMed: 23403047]
47. Margolis DJ, Gupta J, Apter AJ, Ganguly T, Hoffstad O, Papadopoulos M, et al. Filaggrin-2 variation is associated with more persistent atopic dermatitis in African American subjects. J Allergy Clin Immunol. Oct 31.2013 [Epub ahead of print].
48. Esparza-Gordillo J, Weidinger S, Folster-Holst R, Bauerfeind A, Ruschendorf F, Patone G, et al. A common variant on chromosome 11q13 is associated with atopic dermatitis. Nat Genet. 2009; 41:596–601. [PubMed: 19349984]
49. O'Regan GM, Campbell LE, Cordell HJ, Irvine AD, McLean WH, Brown SJ. Chromosome 11q13.5 variant associated with childhood eczema: an effect supplementary to filaggrin mutations. J Allergy Clin Immunol. 2010; 125:170–4. [PubMed: 20109745]
50. Guttman-Yassky E, Suarez-Farinas M, Chiricozzi A, Nograles KE, Shemer A, Fuentes-Duculan J, et al. Broad defects in epidermal cornification in atopic dermatitis identified through genomic analysis. J Allergy Clin Immunol. 2009; 124:1235–44. e58. [PubMed: 20004782]
51. Igawa S, Kishibe M, Honma M, Murakami M, Mizuno Y, Suga Y, et al. Aberrant distribution patterns of corneodesmosomal components of tape-stripped corneocytes in atopic dermatitis and related skin conditions (ichthyosis vulgaris, Netherton syndrome and peeling skin syndrome type B). J Dermatol Sci. 2013; 72:54–60. [PubMed: 23810772]
52. Jarzab J, Filipowska B, Zebracka J, Kowalska M, Bozek A, Rachowska R, et al. Locus 1q21 Gene expression changes in atopic dermatitis skin lesions: deregulation of small proline-rich region 1A. Int Arch Allergy Immunol. 2010; 151:28–37. [PubMed: 19672094]
53. Kim BE, Leung DY, Boguniewicz M, Howell MD. Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. Clin Immunol. 2008; 126:332–7. [PubMed: 18166499]
54. Sevilla LM, Nachat R, Groot KR, Klement JF, Uitto J, Djian P, et al. Mice deficient in involucrin, envoplakin, and periplakin have a defective epidermal barrier. J Cell Biol. 2007; 179:1599–612. [PubMed: 18166659]
55. Fartasch M, Diepgen TL. The barrier function in atopic dry skin. Disturbance of membrane-coating granule exocytosis and formation of epidermal lipids? Acta Derm Venereol Suppl (Stockh). 1992; 176:26–31. [PubMed: 1476030]
56. Werner Y, Lindberg M, Forslind B. Membrane-coating granules in “dry” non-eczematous skin of patients with atopic dermatitis. A quantitative electron microscopic study. Acta Derm Venereol. 1987; 67:385–90. [PubMed: 2448988]

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57. Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A. Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? J Invest Dermatol. 1991; 96:523–6. [PubMed: 2007790]
58. Ishikawa J, Narita H, Kondo N, Hotta M, Takagi Y, Masukawa Y, et al. Changes in the ceramide profile of atopic dermatitis patients. J Invest Dermatol. 2010; 130:2511–4. [PubMed: 20574438]
59. Di Nardo A, Wertz P, Giannetti A, Seidenari S. Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. Acta Derm Venereol. 1998; 78:27–30. [PubMed: 9498022]
60. Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ, et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. J Lipid Res. 2012; 53:2755–66. [PubMed: 23024286]
61. Park YH, Jang WH, Seo JA, Park M, Lee TR, Kim DK, et al. Decrease of ceramides with very long-chain fatty acids and downregulation of elongases in a murine atopic dermatitis model. J Invest Dermatol. 2012; 132:476–9. [PubMed: 22158556]
62. Janssens M, Mulder AA, van Smeden J, Pilgram GS, Wolterbeek R, Lavrijsen AP, et al. Electron diffraction study of lipids in non-lesional stratum corneum of atopic eczema patients. Biochim Biophys Acta. 2013; 1828:1814–21. [PubMed: 23583924]
63. Hara J, Higuchi K, Okamoto R, Kawashima M, Imokawa G. High-expression of sphingomyelin deacylase is an important determinant of ceramide deficiency leading to barrier disruption in atopic dermatitis. J Invest Dermatol. 2000; 115:406–13. [PubMed: 10951276]
64. Kusuda S, Cui CY, Takahashi M, Tezuka T. Localization of sphingomyelinase in lesional skin of atopic dermatitis patients. J Invest Dermatol. 1998; 111:733–8. [PubMed: 9804330]
65. Imokawa G. A possible mechanism underlying the ceramide deficiency in atopic dermatitis: expression of a deacylase enzyme that cleaves the N-acyl linkage of sphingomyelin and glucosylceramide. J Dermatol Sci. 2009; 55:1–9. [PubMed: 19443184]
66. Sawada E, Yoshida N, Sugiura A, Imokawa G. Th1 cytokines accentuate but Th2 cytokines attenuate ceramide production in the stratum corneum of human epidermal equivalents: an implication for the disrupted barrier mechanism in atopic dermatitis. J Dermatol Sci. 2012; 68:25–35. [PubMed: 22884781]
67. Tawada C, Kanoh H, Nakamura M, Mizutani Y, Fujisawa T, Banno Y, et al. Interferon- decreases ceramides with long-chain fatty acids: possible involvement in atopic dermatitis and psoriasis. J Invest Dermatol. Sep 5.2013 [Epub ahead of print].
68. Rebane A, Zimmermann M, Aab A, Baurecht H, Koreck A, Karelson M, et al. Mechanisms of IFN-γ-induced apoptosis of human skin keratinocytes in patients with atopic dermatitis. J Allergy Clin Immunol. 2012; 129:1297–306. [PubMed: 22445417]
69. Niessen CM. Tight junctions/adherens junctions: basic structure and function. J Invest Dermatol. 2007; 127:2525–32. [PubMed: 17934504]
70. Kirschner N, Brandner JM. Barriers and more: functions of tight junction proteins in the skin. Ann N Y Acad Sci. 2012; 1257:158–66. [PubMed: 22671602]
71. Kubo A, Nagao K, Yokouchi M, Sasaki H, Amagai M. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. J Exp Med. 2009; 21(206):2937–46. [PubMed: 19995951]
72. Reefer AJ, Satinover SM, Wilson BB, Woodfolk JA. The relevance of microbial allergens to the IgE antibody repertoire in atopic and nonatopic eczema. J Allergy Clin Immunol. 2007; 120:156–63. [PubMed: 17507082]
73. Wisniewski JA, Agrawal R, Minnicozzi S, Xin W, Patrie J, Heymann PW, et al. Sensitization to food and inhalant allergens in relation to age and wheeze among children with atopic dermatitis. Clin Exp Allergy. 2013; 43:1160–70. [PubMed: 24074334]
74. Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest. 1999; 104:123–33. [PubMed: 10393706]
75. Ohnemus U, Kohrmeyer K, Houdek P, Rohde H, Wladykowski E, Vidal S, et al. Regulation of epidermal tight-junctions (TJ) during infection with exfoliative toxin-negative Staphylococcus strains. J Invest Dermatol. 2008; 128:906–16. [PubMed: 17914452]

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76•. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011; 127:773–86. [PubMed: 21163515] [This article provides both genetic and functional evidence of a role for claudin-1 in the impairment of tight junctions in humans with AD.]

77. De Benedetto A, Slifka MK, Rafaels NM, Kuo IH, Georas SN, Boguniewicz M, et al. Reductions in claudin-1 may enhance susceptibility to herpes simplex virus 1 infections in atopic dermatitis. J Allergy Clin Immunol. 2011; 128:242–6. [PubMed: 21489616]

78. Braff MH, Bardan A, Nizet V, Gallo RL. Cutaneous defense mechanisms by antimicrobial peptides. J Invest Dermatol. 2005; 125:9–13. [PubMed: 15982297]

79. Howell MD, Gallo RL, Boguniewicz M, Jones JF, Wong C, Streib JE, et al. Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. Immunity. 2006; 24:341–8. [PubMed: 16546102]

80. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med. 2002; 347:1151–60. [PubMed: 12374875]

81. Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, et al. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. J Immunol. 2003; 171:3262–9. [PubMed: 12960356]

82. Rieg S, Steffen H, Seeber S, Humeny A, Kalbacher H, Dietz K, et al. Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. J Immunol. 2005; 174:8003–10. [PubMed: 15944307]

83. Howell MD, Wollenberg A, Gallo RL, Flaig M, Streib JE, Wong C, et al. Cathelicidin deficiency predisposes to eczema herpeticum. J Allergy Clin Immunol. 2006; 117:836–41. [PubMed: 16630942]

84. Hata TR, Kotol P, Boguniewicz M, Taylor P, Paik A, Jackson M, et al. History of eczema herpeticum is associated with the inability to induce human beta-defensin (HBD)-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. Br J Dermatol. 2010; 163:659–61. [PubMed: 20545685]

85. Ballardini N, Johansson C, Lilja G, Lindh M, Linde Y, Scheynius A, et al. Enhanced expression of the antimicrobial peptide LL-37 in lesional skin of adults with atopic eczema. Br J Dermatol. 2009; 161:40–7. [PubMed: 19309368]

86. Asano S, Ichikawa Y, Kumagai T, Kawashima M, Imokawa G. Microanalysis of an antimicrobial peptide, beta-defensin-2, in the stratum corneum from patients with atopic dermatitis. Br J Dermatol. 2008; 159:97–104. [PubMed: 18476959]

87. Gambichler T, Skrygan M, Tomi NS, Othlinghaus N, Brockmeyer NH, Altmeyer P, et al. Differential mRNA expression of antimicrobial peptides and proteins in atopic dermatitis as compared to psoriasis vulgaris and healthy skin. Int Arch Allergy Immunol. 2008; 147:17–24. [PubMed: 18446049]

88. Glaser R, Meyer-Hoffert U, Harder J, Cordes J, Wittersheim M, Kobliakova J, et al. The antimicrobial protein psoriasin (S100A7) is upregulated in atopic dermatitis and after experimental skin barrier disruption. J Invest Dermatol. 2009; 129:641–9. [PubMed: 18754038]

89. Harder J, Dressel S, Wittersheim M, Cordes J, Meyer-Hoffert U, Mrowietz U, et al. Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury. J Invest Dermatol. 2010; 130:1355–64. [PubMed: 20107483]

90. Kisich KO, Carspecken CW, Fieve S, Boguniewicz M, Leung DY. Defective killing of Staphylococcus aureus in atopic dermatitis is associated with reduced mobilization of human beta-defensin-3. J Allergy Clin Immunol. 2008; 122:62–8. [PubMed: 18538383]

91. Kim E, Lee JE, Namkung JH, Kim PS, Kim S, Shin ES, et al. Single nucleotide polymorphisms and the haplotype in the DEFB1 gene are associated with atopic dermatitis in a Korean population. J Dermatol Sci. 2009; 54:25–30. [PubMed: 19135873]

92. Prado-Montes de Oca E, Garcia-Vargas A, Lozano-Inocencio R, Gallegos-Arreola MP, Sandoval-Ramirez L, Davalos-Rodriguez NO, et al. Association of beta-defensin 1 single nucleotide

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polymorphisms with atopic dermatitis. Int Arch Allergy Immunol. 2007; 142:211–8. [PubMed: 17108702]

93. Howell MD, Boguniewicz M, Pastore S, Novak N, Bieber T, Girolomoni G, et al. Mechanism of HBD-3 deficiency in atopic dermatitis. Clin Immunol. 2006; 121:332–8. [PubMed: 17015038]
94. Howell MD, Novak N, Bieber T, Pastore S, Girolomoni G, Boguniewicz M, et al. Interleukin-10 downregulates anti-microbial peptide expression in atopic dermatitis. J Invest Dermatol. 2005; 125:738–45. [PubMed: 16185274]
95. Alase A, Seltmann J, Werfel T, Wittmann M. Interleukin-33 modulates the expression of human beta-defensin 2 in human primary keratinocytes and may influence the susceptibility to bacterial superinfection in acute atopic dermatitis. Br J Dermatol. 2012; 167:1386–9. [PubMed: 22803572]
96. Komatsu N, Saijoh K, Kuk C, Liu AC, Khan S, Shirasaki F, et al. Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. Exp Dermatol. 2007; 16:513–9. [PubMed: 17518992]
97. Komatsu N, Saijoh K, Toyama T, Ohka R, Otsuki N, Hussack G, et al. Multiple tissue kallikrein mRNA and protein expression in normal skin and skin diseases. Br J Dermatol. 2005; 153:274–81. [PubMed: 16086736]
98. Voegeli R, Rawlings AV, Breternitz M, Doppler S, Schreier T, Fluhr JW. Increased stratum corneum serine protease activity in acute eczematous atopic skin. Br J Dermatol. 2009; 161:70–7. [PubMed: 19416247]
99. Vasilopoulos Y, Cork MJ, Murphy R, Williams HC, Robinson DA, Duff GW, et al. Genetic association between an AACC insertion in the 3'UTR of the stratum corneum chymotryptic enzyme gene and atopic dermatitis. J Invest Dermatol. 2004; 123:62–6. [PubMed: 15191543]
100. Hubiche T, Ged C, Benard A, Leaute-Labreze C, McElreavey K, de Verneuil H, et al. Analysis of SPINK 5, KLK 7 and FLG genotypes in a French atopic dermatitis cohort. Acta Derm Venereol. 2007; 87:499–505. [PubMed: 17989887]
101. Buddenkotte J, Stroh C, Engels IH, Moormann C, Shpacovitch VM, Seeliger S, et al. Agonists of proteinase-activated receptor-2 stimulate upregulation of intercellular cell adhesion molecule-1 in primary human keratinocytes via activation of NF-kappa B. J Invest Dermatol. 2005; 124:38–45. [PubMed: 15654951]
102. Steinhoff M, Neisius U, Ikoma A, Fartasch M, Heyer G, Skov PS, et al. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J Neurosci. 2003; 23:6176–80. [PubMed: 12867500]
103. Morizane S, Yamasaki K, Kajita A, Ikeda K, Zhan M, Aoyama Y, et al. TH2 cytokines increase kallikrein 7 expression and function in patients with atopic dermatitis. J Allergy Clin Immunol. 2012; 130:259–61. e1. [PubMed: 22521249]
104. Cheng T, Tjabringa GS, van Vlijmen-Willems IM, Hitomi K, van Erp PE, Schalkwijk J, et al. The cystatin M/E-controlled pathway of skin barrier formation: expression of its key components in psoriasis and atopic dermatitis. Br J Dermatol. 2009; 161:253–64. [PubMed: 19438477]
105. Kim N, Bae KB, Kim MO, Yu DH, Kim HJ, Yuh HS, et al. Overexpression of cathepsin S induces chronic atopic dermatitis in mice. J Invest Dermatol. 2012; 132:1169–76. [PubMed: 22170489]
106. Tsukuba T, Okamoto K, Okamoto Y, Yanagawa M, Kohmura K, Yasuda Y, et al. Association of cathepsin E deficiency with development of atopic dermatitis. J Biochem. 2003; 134:893–902. [PubMed: 14769879]
107. Hvid M, Johansen C, Deleuran B, Kemp K, Deleuran M, Vestergaard C. Regulation of caspase 14 expression in keratinocytes by inflammatory cytokines--a possible link between reduced skin barrier function and inflammation? Exp Dermatol. 2011; 20:633–6. [PubMed: 21539619]
108. Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet. Jun. 2000; 25(2):141–2. [PubMed: 10835624]
109. Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C, et al. Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med. May 11. 2009; 206(5):1135–47. [PubMed: 19414552]

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110. Briot A, Lacroix M, Robin A, Steinhoff M, Deraison C, Hovnanian A. Par2 inactivation inhibits early production of TSLP, but not cutaneous inflammation, in Netherton syndrome adult mouse model. J Invest Dermatol. 2010; 130:2736–42. [PubMed: 20703245]

111. Kusunoki T, Okafuji I, Yoshioka T, Saito M, Nishikomori R, Heike T, et al. SPINK5 polymorphism is associated with disease severity and food allergy in children with atopic dermatitis. J Allergy Clin Immunol. 2005; 115:636–8. [PubMed: 15753919]

112. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R, et al. Gene polymorphism in Netherton and common atopic disease. Nat Genet. 2001; 29:175–8. [PubMed: 11544479]

113. Folster-Holst R, Stoll M, Koch WA, Hampe J, Christophers E, Schreiber S. Lack of association of SPINK5 polymorphisms with nonsyndromic atopic dermatitis in the population of Northern Germany. Br J Dermatol. 2005; 152:1365–7. [PubMed: 15949016]

114. Weidinger S, Baurecht H, Wagenpfeil S, Henderson J, Novak N, Sandilands A, et al. Analysis of the individual and aggregate genetic contributions of previously identified serine peptidase inhibitor Kazal type 5 (SPINK5), kallikrein-related peptidase 7 (KLK7), and filaggrin (FLG) polymorphisms to eczema risk. J Allergy Clin Immunol. 2008; 122:560–8. [PubMed: 18774391]

115. Stout TE, McFarland T, Mitchell JC, Appukattan B, Stout TJ. Recombinant filaggrin is internalized and processed to correct filaggrin deficiency. J Invest Dermatol. 2014; 134:423–9. [PubMed: 23792461]

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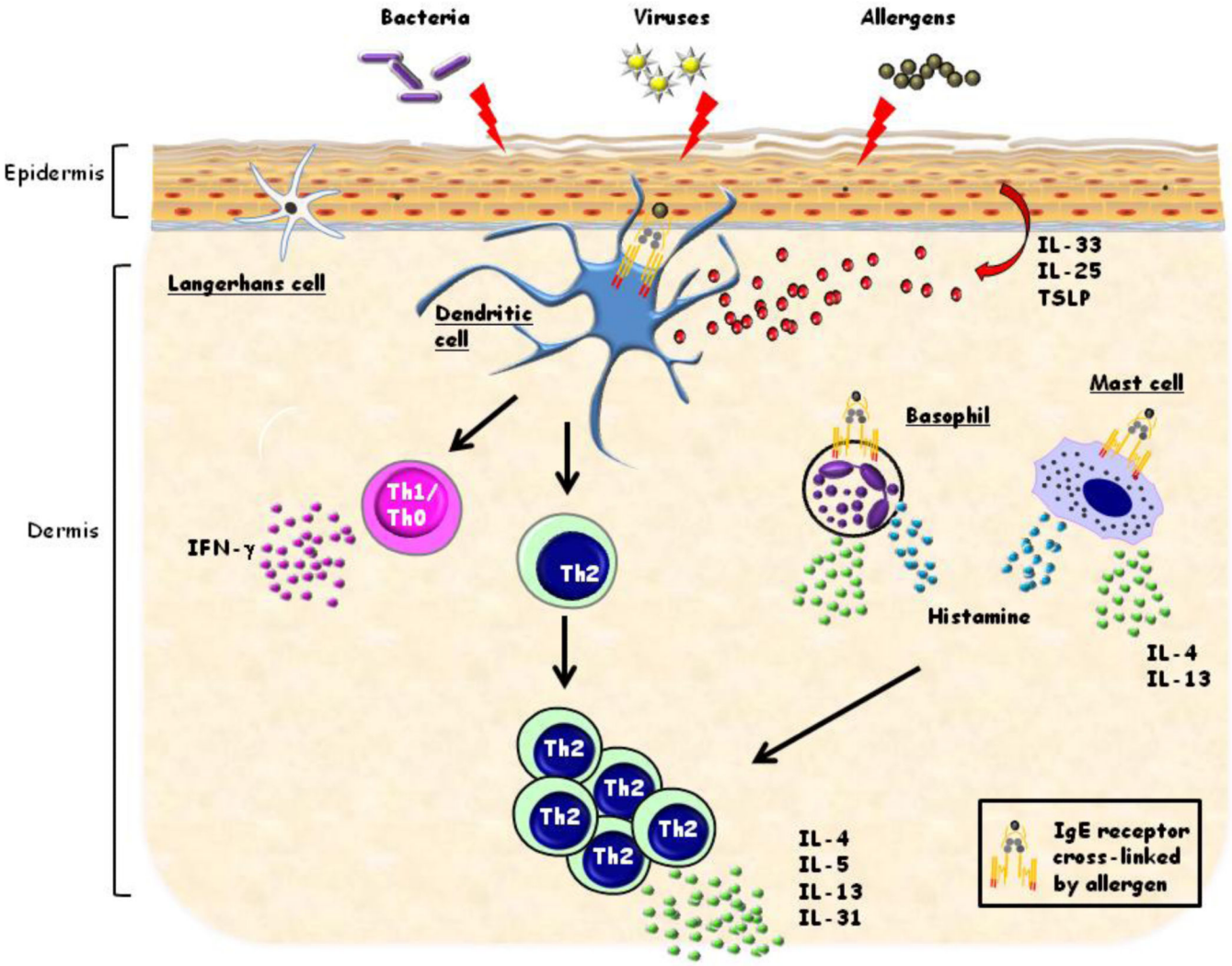

Figure 1. Schematic of the Immune Response in AD Skin Lesions

Defects in the epithelial barrier provide a portal of entry for microbial products and allergens. In conjunction, these antigens trigger the innate response through induction of cytokines from the epithelium. Together, these events orchestrate the induction of Th2 cells which secrete IL-4, IL-5, IL-13 and IL-31, production of IgE, and activation of basophils and mast cells. This process is highly dynamic and evolves to include hetereogeneous T-cell types including Th1 cells.

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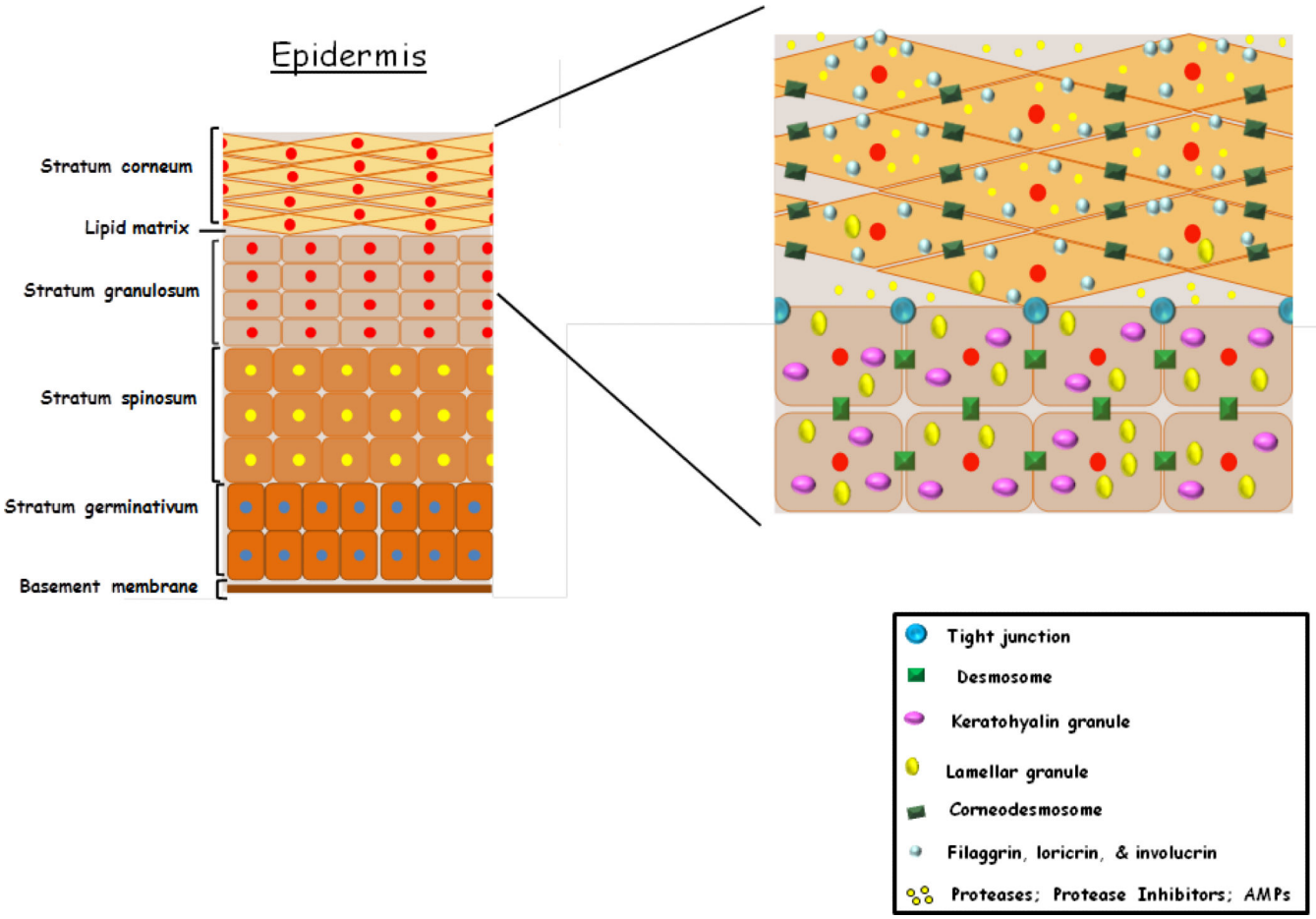

Figure 2. Structural and Immune Components Contributing to Barrier Defects in AD

The expanded view provides a schematic representation of the distribution of structural proteins and components of the innate immune system in the uppermost layers of the epidermis.

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Table 1

Summary of Skin Barrier Defects in Atopic Dermatitis.

Factor/Molecule	Location	Function	Molecular Defect	Relevance to AD
Filaggrin	Cytoskeleton of keratinocytes in the stratum corneum	Formation of corneocytes; Maintains physical strength; Minimizes entry of foreign antigens; Reduces TEWL	↓ expression of filaggrin protein [*5] resulting from: (1) Loss-of-function mutations in *FLG* gene [*7-9,14]; (2) Lower intragenic copy number in *FLG* gene [*16]; (3) ↑ methylation of *FLG* gene [*17] Modulation by Th2 cytokines [†§37-43]	Disruption of skin barrier arising from: Disorganized keratin filaments & abnormal/ impaired lipid bilayer [¶†18, 20]; Altered acidification pathways [†20]; ↓ hydration and ↑ TEWL [*22] ↑cytokine levels in skin [*#23,24]
Filaggrin-like proteins (filaggrin-2, hornerin)	Cytoskeleton of keratinocytes in the stratum corneum	Presumed similar functions as filaggrin	↓ Protein levels in skin [*45, 46]; Premature stop codon mutations in *FLG2* gene [*47]; SNPs in *HRNR* gene [*48, 49]	*Similar to those for filaggrin defects*
Natural Moisturizing Factors (Urocanic Acid, Pyrrolidine Carboxylic Acid)	Stratum corneum	Maintain moisture and pH	↓ Protein levels in skin [*21, 26, 27]	*Disruption of skin physiology; ↑susceptibility to infection*
Other structural/scaffold proteins (Loricrin, involucrin, envoplakin, periplakin)	Cornified envelope	Reinforcement of the cornified envelope	↓ RNA or protein levels in skin [*#50, 52, 53, 54]	Hyperkeratosis & ↑cellular infiltrates; ↓ lipid content of skin
Corneodesmosomal proteins (desmoglein-1, desmocolin-1, corneodesmosin)	Corneodesmosome	Attachment of adjacent corneocytes	↓ Protein levels in skin [*45] Modulation by Th2 cytokines [§37, 53]	Reduced compaction of corneocytes in stratum corneum [*50]
Lipids (ceramides, cholesterol, free fatty acids)	Lipid bilayer and lamellar granules	Strengthen epidermal barrier; Maintain hydration status	Decreased levels in skin [*57-59]; Disturbed or incomplete lamellar body maturation and extrusion [*55, 56]; Shorter chain length lipids [*#60,61]; Increased expression of lipid-processing enzymes [*63-65]; Modulation by pro-inflammatory cytokines including IL-4 and IL-6 [†66]	Abnormal lipid organization in skin [*60, 62]
Tight junction proteins (claudins, occludens)	Stratum corneum and stratum granulosum	Strengthen the epidermal barrier; Reduce TEWL	Reduced RNA and protein levels of claudins in skin [*§76, 77]; Disruption of tight junctions by environmental proteases [§#74, 75]	*↑susceptibility to infection; ↑antigen entry and consequent immune dysregulation*
Anti-microbial peptides (AMPs) (β-defensins, cathelicidins)	Stratum corneum and stratum granulosum	Lyse bacteria, fungi and viruses by permeabilizing microbial membranes	↓ production/mobilization from lamellar granules; [*§79-90]; Modulation by Th2 cytokines [*#§†79, 81, 93, 94]; SNPs in β-defensin 1 gene [*91, 92].	*↑ microbial colonization and infection*
Proteases (kallikreins; cathepsins; caspase-14)	Stratum corneum and stratum granulosum	Maintain desquamation; Regulate levels of structural proteins, lipid-processing enzymes and AMPs	Aberrant expression / ↑enzymatic activity in skin [*§96-98; 103, 104, 107]; Mutations linked with AD [*99, 100]	*Abnormal desquamation. ↑breakdown of structural proteins, lipid-processing enzymes and AMPs*

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Factor/Molecule	Location	Function	Molecular Defect	Relevance to AD
Protease inhibitors (Lymphoepithelial kazal-type related inhibitor)	Stratum corneum and stratum granulosum	Maintain desquamation; Regulate protease levels	Loss-of-function mutation in *SPINK5* [*108]; SNPs in *SPINK5* [*111-114]	Causes severe AD associated with Netherton syndrome [*]; Thinning of stratum corneum and ↑ TSLP [*§#109, 110]

Italics denote the predicted effect on skin, though this has not been demonstrated experimentally. *TEWL*, trans-epidemal water loss.

[*] Reported in AD patients

[¶] ichthyosis vulgaris patients.

[†] Reported in *ex vivo* skin models

[§] epithelial/keratinocyte cultures.

[#] Reported in animal models.

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