Published in final edited form as:
*J Allergy Clin Immunol*. 2014 October ; 134(4): 781–791.e1. doi:10.1016/j.jaci.2014.05.048.

# Mechanisms of abnormal lamellar body secretion and the dysfunctional skin barrier in atopic dermatitis

**Peter M. Elias, MD** and **Joan Wakefield**
Dermatology Service, Veterans Affairs Medical Center, and Department of Dermatology, University of California, San Francisco, CA

## Abstract

We review here how diverse inherited and acquired abnormalities in epidermal structural and enzymatic proteins converge to produce defective permeability barrier function and antimicrobial defense in AD. Although best known are mutations in *filaggrin (FLG)*, mutations in other member of the fused S-100 family of proteins (i.e., hornerin [hrn] and filaggrin 2 [flg-2]); the cornified envelope precursor (e.g., SPRR3); mattrin, encoded by *Tmem79*, which regulates the assembly of lamellar bodies; *SPINK5*, which encodes the serine protease inhibitor, LEKTI1; and the fatty acid transporter, FATP4, have all been linked to AD. Yet, these abnormalities often only predispose to AD; additional acquired stressors that further compromise barrier function; e.g., psychological stress, a low ambient humidity, or high pH surfactants, often are required to trigger disease. Th2 cytokines can also compromise barrier function by downregulating expression of multiple epidermal structural proteins, lipid synthetic enzymes and antimicrobial peptides. All of these inherited and acquired abnormalities converge on the lamellar body secretory system, producing abnormalities in lipid composition, secretion and/or extracellular lamellar membrane organization, as well as in antimicrobial defense. Finally, we briefly review therapeutic options that address this new pathogenic paradigm.

### Keywords

antimicrobial peptides; atopic dermatitis; barrier function; ceramides; cytokines; filaggrin; kallikreins; lamellar bodies; lipid composition; pH; serine protease inhibitors; Th2 cells

## INTRODUCTION

### Basis for the Permeability Barrier in Normal Skin

7The epidermis generates a set of protective/defensive functions, mediated by its unique differentiation end-product, the stratum corneum (SC) [1, 2]. As they migrate apically,

Address correspondence to: Peter M. Elias, MD, Dermatology Service (190), VA Medical Center, 4150 Clement Street, San Francisco, CA 94121, TEL: (415) 750-2091, FAX: (415) 750-2106, eliasp@derm.ucsf.edu.

The authors state no conflicts of interest.

keratinocytes acquire a series of differentiation-specific proteins [3–5] and lipids [6, 7] until cornification occurs. The SC comprises vertical stacks of anucleate corneocytes, embedded in an expanded extracellular matrix, replete with multiple stacks of broad, planar lamellar bilayers, enriched in ceramides, cholesterol, and free fatty acids (FFA) [8]. These lamellar arrays of hydrophobic lipid species impede both the outward movement of water, and the inward bombardment of noxious environmental allergens and pathogens. A unique organelle, the epidermal lamellar body, delivers these lipids to the SC interstices as their precursors (e.g., glucosylceramides and phospholipids), along with a set of hydrolytic 'lipid processing' enzymes, including β-glucocerebrosidase, acidic sphingomylinase, secretory phospholipase $A_2$ and steroid sulfatase [9] (Fig. 1). These enzymes generate a family of ceramides (Cer), essential and non-essential free fatty acids (FFA), as well as much of the cholesterol that is required for the supramolecular organization of these non-polar lipid species into mature lamellar membrane structures [10]. In parallel, lamellar body-derived, desquamatory proteases and their inhibitors initiate the orderly digestion of corneodesmosomes (= transient intercellular rivets that connect adjacent corneocytes), a process that eventually leads to the desquamation of corneocytes from the skin surface [11, 12] (Fig. 1). Finally, at least two antimicrobial peptides, human β-defensin 2 and the carboxyterminal cathelicidin peptide, LL-37, are delivered to the SC intercellular domains through secretion of lamellar body contents [13–15]. In fact, the *permeability barrier* and the *antimicrobial barrier* share many features that impede the colonization and invasion of pathogenic organisms, while simultaneously encouraging colonization by non-pathogenic 'normal' flora [16]. Thus, the physicochemical characteristics of the SC; niche occupancy by resident normal flora; and secreted factors from the normal flora contribute to cutaneous antimicrobial defense. Because these two barriers share so many structural and biochemical features, perturbations in one function inevitably modify the other in parallel [15, 17]. Thus, the epidermal lamellar body is a multifunctional organelle, whose contents influence not only permeability barrier status, but also SC cohesion/desquamation and antimicrobial defense.

**The Tight Junction (TJ) Controversy—**How should we interpret an ever-expanding literature that proclaims a potential role for TJ in normal permeability barrier function [18, 19], as well as a potential role for abnormal TJ function in AD [20]? We will attempt to navigate this heavily invested subject as follows: First, complex TJ structures, such as those found in the kidney and gastrointestinal tract, do not occur in adult keratinizing epithelia [21]. Second, with the exception of these structures in renal collecting tubules, where they comprise multitiered sites of membrane fusion ('zonulae occludentes'), TJ provide a relatively poor barrier against paracellular water movement. In the author's opinion, confusion in the skin-related literature has occurred because 'TJ *proteins*' are widely equated with 'TJ' [18, 19]. Doubtless, the apical-lateral plasma membranes of cells in the outer stratum granulosum of normal epidermis are heavily decorated with multiple TJ proteins, which form 'kissing points'; i.e., 'maculae occludentes' [21]. However, as noted above, these focal attachments do not comprise true zonulae occludentes, as occur in tubular epithelia. The most compelling evidence that these putative TJ play no direct role in the paracellular water barrier is that removal of SC lipids by gentle, external lipid solvent treatment *completely abrogates* the permeability barrier [22]. While this observation likely also excludes a possible 'back-up' role for TJ-like structures in the *water* barrier, it still remains possible that these incomplete

structures interdict the passage of larger xenobiotes, particularly when the overlying lipid-based barrier becomes defective, as occurs in AD.

Yet, these structures, though insufficient to contribute to the water barrier, are nonetheless critical for the development of barrier competence. Transgenic knock-out of the key TJ protein, claudin 1, results in a fatal, post-natal permeability barrier abnormality [23]. Indeed, replete TJ are present early in epidermal development, but they become functionally incompetent later in fetal life in parallel with establishment of the lipid-based barrier [24]. An acquired reduction in the expression of the TJ protein, claudin 1, has been reported in AD [20], and occludin protein levels decline in FLG-deficient human epidermis [25]. It is possible that abnormalities in TJ proteins could result from the Th2 dominant milieu in AD, which simultaneously downregulates many other epidermal differentiation-linked proteins (see below).

Since adult epidermis does not generate the types of complex zonulae occludentes necessary to impede paracellular water movement, attention should be focused instead on the possible function(s) of the incomplete junctions (maculae occludentes) in normal epidermis; and how acquired defects in these focal connections could contribute to AD pathogenesis. These structures likely perform important ‘fence functions’ in adult epidermis, including polarizing the direction of lamellar body secretion towards the apex of the outermost granular layer [26].

**Barrier Dysfunction in AD and Other Atopic Disorders—**During the pre-genotype era, we and others proposed that the permeability barrier abnormality in AD is not merely an epiphenomenon, but rather the potential ‘driver’ of inflammation in AD (i.e., an ‘outside-to-inside’ view of disease pathogenesis) [27, 28], because i) the extent of the permeability barrier abnormality parallels severity of disease phenotype [29, 30]; ii) clinically-uninvolved skin sites display significant barrier abnormalities [30]; and iii) sustained barrier abnormalities, regardless of cause, inevitably stimulate a pro-inflammatory cytokine cascade that ‘recruits’ characteristic, disease-specific immunophenotypes [31, 32]. Recent studies have shown that the cutaneous barrier abnormality is not only critical for the development of AD, but also other allergic disorders, including asthma, allergic rhinitis, and food allergies [33].

The barrier abnormality in AD leads to an increase in pH that activates serine proteases (kallikreins, KLK) in the outer epidermis, with widespread downstream and upstream consequences, as shown in Fig. 2. Yet, these mechanisms also stimulate a series of metabolic responses aimed at restoring permeability barrier homeostasis. Briefly, increased TNFα stimulates differentiation [34], while increased IL-1α enhances epidermal lipid synthesis [35]. Nerve growth factor (NGF) and amphiregulin (AR) stimulate epidermal DNA synthesis; and several cytokines enhance antimicrobial peptide activation/production [36].

**Related and Unrelated Mutations Impact Barrier Function in AD—**Recent molecular genetic studies have fortified this ‘outside-to-inside-back- to-outside’ view of disease pathogenesis, because most of the recently-identified mutations associated with AD modify structural and enzymatic proteins that are required for normal barrier function.

**Filaggrin:** The initial molecular genetic evidence that a primary structural abnormality underlies the pathogenesis of AD derives from the strong association of loss-of-function mutations in the gene encoding *filament aggregating protein* (filaggrin, FLG) in AD [37–39]. Up to 50% of certain northern European kindreds, and a substantial proportion of Asians, with AD reveal either single or double allele, loss-of-function mutations in the gene encoding FLG. Although more than 40 different *FLG* mutations have now been associated with AD [37], four predominate in northern and central Europeans [40, 41]. The initial product of FLG translation is pro-FLG, a large, histidine-rich, highly cationic phosphoprotein, consisting of ten to twelve FLG repeats, connected by peptide segments enriched in hydrophobic amino acids [42, 43]. Pro-FLG contains an amino-terminal sequence, including a calcium-binding A domain as well as a B domain of uncertain function. During cornification in normal, non-atopic humans, pro-FLG is dephosphorylated and proteolytically processed to FLG monomers [42]. Then, as the water content of the SC declines in the mid-to-outer stratum corneum, FLG detaches from the cornified envelope, followed by its C-terminal proteolysis by caspase 14, bleomycin hydrolase, and other hydrolases into its constituent amino acids [44]. These amino acids subsequently are further deiminated into polycarboxylic acids that account in part for SC hydration and acidification [45] (Fig. 3).

Decreased FLG expression results in a paucity of keratohyalin granules, a hallmark of ichthyosis vulgaris (IV) [46], the *forme fruste* of AD [47]. *Flg* mutations exhibit an allele-dose effect, wherein heterozygous patients with IV show diminished FLG expression and a mild phenotype, as well as abnormalities in surface pH, hydration, and barrier function [25] (Fig. 3). But IV patients with homozygous and compound heterozygous *FLG* mutations exhibit more severe scaling [40, 48], and more pronounced abnormalities in stratum corneum structure and function [25] (Fig. 4), as well as a further propensity to develop AD [37, 40]. Yet importantly, a substantial proportion of these double-allele IV patients still do not exhibit inflammation (i.e., AD), emphasizing the role of exogenous (acquired) stressors in AD pathogenesis (see below).

An acquired reduction in epidermal FLG expression also occurs in AD, independent of mutation status [49], due in part to Th2-induced down-regulation of a broad range of proteins associated with epidermal differentiation, including FLG [5051]. Moreover, IgE from AD patients auto-reacts against a variety of keratinocyte antigens, suggesting yet another ‘vicious cycle’ in AD [52]. Thus, primary inherited barrier abnormalities in AD ultimately stimulate downstream paracrine mechanisms that likely further compromise permeability barrier function, completing an additional potential ‘outside-inside-outside’ pathogenic loop in AD [53] (Fig. 2).

**Other Fused S-100 Proteins (Hornerin and Filaggrin-2):** The prevalence of FLG mutations in AD patients, though quite high in populations of northern European descent [37], does not account for many cases of AD in such northerners. It can be further assumed that almost all cases of AD in other populations will prove to be associated with other inherited abnormalities that compromise epidermal barrier function. Recent studies suggest an association of AD with mutations in two other members of the fused S-100 proteins; i.e., hornerin (hrn) and filaggrin 2 (Flg-2) [54, 55]. Interestingly, *FLG-2* mutations are linked to AD in African-Americans [56, 57]. While filaggrin, hornerin, and FLG-2 are all differentiation-

specific components of the corneocyte envelope [58–62], the specific functions of both hornerin and FLG-2 in normal epidermis remain unknown (Table 1). Hence, how defects in these proteins contribute to a putative barrier abnormality in AD also remains uncertain.

SPRR3 is a cornified envelope (CE) precursor protein that is virtually undetectable in normal skin [63]. Several types of mutations in SPRR3, including an extra 24 base pair defect in the central domain, as well as additional in-frame deletions and insertions [64] have been associated not only with AD [65], but also with asthma [66]. These mutations result in expression of SPRR3 at higher than normal levels in AD [64, 65], likely impacting barrier function through production of a CE scaffold that impairs the supramolecular organization of lamellar body-derived lipids into normal bilayer structures (Table 1). Ultrastructural studies show defective, thinner-than-normal CEs in AD, with decreased extracellular lipids and a poorly cohesive SC, associated with deficiencies not only in SPRR, but also in several other CE precursors in AD, perhaps due to a broad Th2-stimulated down-regulation of these proteins [6750, 51].

**TMEM79:** Very recent studies have identified non-sense and mis-sense mutations in the gene, *TMEM79*, which encodes the protein, mattrin, in some Irish AD patients who lack *FLG* mutations [68]. Mutations in the murine orthologue of this gene account for the flaky tail (*ma/ma)* strain of mice, which develop a spontaneous AD-like dermatitis [69]. Mattrin localizes to the cytosol, and more specifically within the trans-Golgi network in the outermost cells of the granular layer [68, 69]. Reductions in mattrin levels block the secretion of lamellar body contents, including desquamatory proteases, antiproteases [69], and likely lamellar-derived lipids. Indeed, defective lipid secretion has been demonstrated in flaky tail mice, bearing the *mattrin* mutation [70]. Nonetheless, the association of this mutation with AD eloquently demonstrates that not only inherited deficiencies in structural proteins, but also that mutations which impair the delivery of lamellar body contents can predispose to disease.

**Protease/Antiprotease Expression:** Inherited abnormalities that result in excessive serine protease (SP) (kallikreins, KLK) activity predisposes to severe AD, and more importantly, they provide unique insights into the pathogenesis of AD [71, 72] (Fig. 5). The most compelling demonstration for the role of excess SP activity in the pathogenesis of AD comes from Netherton syndrome (NS), an autosomal recessive disorder due to loss-of-function mutations in *SPINK5*, the gene encoding the SP inhibitor, lymphoepithelial Kazal-type trypsin inhibitor type 1 (LEKTI 1) [73]. NS is characterized by a severe AD-like dermatosis, mucosal atopy, and anaphylactic reactions to food antigens. The extent of residual LEKTI expression in humans with NS correlates inversely with excess KLK activity within the outer epidermis [74], and unrestricted KLK activity provokes a severe permeability barrier defect, as well as dramatic thinning of the SC. Both defects can be attributed to KLK-dependent degradation of lipid-processing enzymes and corneodesmosome-constituent proteins, respectively [74]. KLK-mediated degradation of the enzymes contributes to the depletion of Cer, a characteristic lipid abnormality in AD [75, 76] (see also below). Likewise, transgenic mice that over-express human KLK7 display a severe AD-like dermatosis [77]. In NS, and likely also in AD, one of these KLKs; i.e., KLK5, or the

SC tryptic enzyme, binds to the protease activated receptor, type 2 (PAR2), stimulating NFκB-dependent production of the pro-Th2 cytokine, thymic stromal lymphopoietin (TSLP) [78].

As a result of these divergent inherited associations, a broad view is emerging that virtually any inherited abnormality that leads to a sustained barrier abnormality can predispose to AD. For example, note the association of AD with loss-of-function mutations in the *fatty acid transporter*, *FATP4*, in patients with ichthyosis prematurity syndrome [79]. Moreover, many patients with other inherited ichthyoses frequently report severe pruritus [80], although the inflammatory infiltrate in these patients (with the exception of NS) has not yet been characterized (Table 1). Yet, it would not be unreasonable then to query why diseases like psoriasis, which exhibit a well-known barrier abnormality [81], do not develop a Th-2-like immunophenotype. A perhaps too-simple explanation might be that allergen access is impeded by the tenacious scale in psoriasis.

## How Unrelated Mutations in Epidermal Proteins Converge on the Lamellar Body Secretory System to Provoke a Barrier Abnormality in AD

We have shown that both reductions and loss of the cytosolic protein, filaggrin, lead to an extracellular permeability barrier defect, both in filaggrin-deficient IV [25], and in murine models of AD [70, 82]. In all these settings, water loss accelerates via an extra- (para-) cellular pathway; i.e., through the extracellular matrix. The link between a defect in the cytosolic protein, Flg, and the extracellular permeability defect was clarified in patients with IV (Figs. 4A&B) [25]. Both single- and double-allele patients demonstrate retraction of cytosolic keratin filaments into a perinuclear shell around nuclei of the stratum granulosum (Fig. 4C). This cytoskeletal abnormality appears to impact two cellular processes. First, it results in incomplete loading of cargo into nascent lamellar bodies, evidenced by ‘empty’ micro-vesicles within these organelles (Figs. 4D&E). The resulting deposition of non-lamellar contents in the intercellular spaces then leads to focal defects in the extracellular lamellar bilayer system (Figs. 4F&G), contributing to defective barrier function in IV. Second, the cytoskeletal abnormality impairs secretion of lamellar bodies, resulting in their partial entombment within corneocytes [25, 70] (Fig. 5). This latter pathogenic sequence is similar to that seen in patients with mutations in keratins 1 or 10, where cytoskeletal retraction results in entombment of lamellar bodies, and a paucity of lamellar bilayers [83].

Once inflammation is established; i.e., as IV transitions into AD, the pH of the SC increases still further, sufficient to activate a family of KLK in the outer epidermis [84]. KLK activation has multiple negative consequences [72], including: i) the degradation of both corneodesmosomes, leading to a poorly cohesive SC, and ii) the destruction of extracellular lipid processing enzymes, β-glucocerebrosidase and acidic sphingomylinase [85]. Loss of these two ceramide-generating enzymes (as well as their reduced activity at a high pH) results in failure of lamellar bilayer formation and maturation [25, 82], exactly as occurs in NS [74]. A third downstream consequence of increased SP activity is generation of the primary cytokines, IL-1α and IL-1β from their 33kDa pro-forms in human SC [86], which are stored in large quantities in the cytosol of corneocytes [87, 88]. This putative pH-induced increase in KLK activity generates the active, 17kDa forms of these cytokines, the first step

in the cytokine cascade in AD, which includes downstream production of several additional cytokines, growth factors, chemokines, and adhesion molecules [31, 89, 90], including the TSLP-Th2 cytokine network, described above [78] (Fig. 2).

Excess KLK also activates the PAR2 receptor, which localizes to the plasma membrane of granular cells [74]. Binding is followed by internalization of the PAR2 receptor, which then down-regulates lamellar body secretion, effectively entombing these organelles within the corneocyte cytosol [91, 92]. Thus, even without allergen exposure, an Th2 immunophenotype likely can occur in AD, as described for NS [78] (Fig. 5)! Conversely, applications of either KLK or PAR2 inhibitors, or just acidification of the SC alone [9394], prevents both the destructive effects of excess KLK activity and PAR2 internalization, normalizing lamellar body secretion and permeability homeostasis [91, 95]. These studies demonstrate how multiple, pH-initiated steps in the secretion and post-secretory processing of lamellar body contents leads to AD (Fig. 6).

## Stressors That Further Aggravate Barrier Dysfunction Can Trigger AD (Fig. 5)

**Alkaline soaps:** In ichthyosis vulgaris (IV), even double-allele *FLG* mutations do not always suffice to provoke inflammation [25, 39, 96], but certain stressors can further aggravate the barrier abnormality; i.e., by provoking an incremental increase in the pH of the SC, leading to a further amplification of SP activity [72]. Such a barrier-dependent increase in pH (and SP activity) likely accounts for the precipitation of AD following the use of *neutral-to-alkaline soaps*, a well-known exogenous stressor of clinical AD [97, 98].

**Reduced Humidity as a Stressor:** Prolonged exposure to a *reduced environmental humidity*, as occurs in radiant-heated homes in temperate climates during the winter, is also a well-known risk factor for AD [99]. Under these conditions, transcutaneous water loss accelerates across a defective SC, aggravating the underlying permeability barrier abnormality, while also amplifying cytokine signaling of inflammation [72]. Because FLG proteolysis is regulated by changes in external humidity [45], sustained reductions in environmental relative humidities likely further deplete residual FLG in single-allele *FLG*-deficient patients with AD.

Finally, sustained *psychological stress (PS)* aggravates permeability barrier function in otherwise normal humans [100, 101] and mice [102, 103]. PS is also a widely-acknowledged precipitant of AD, but in the case of PS, however, the likely mechanism differs from either high pH surfactant use or decreased environmental humidities. Increased stress in *experimental animals* induces an increase in endogenous glucocorticoids (GC), which in turn alters permeability barrier homeostasis, SC cohesion and epidermal antimicrobial defense [15, 103, 104]. The central role of GC has been demonstrated in two ways: first, exogenous systemic or topical GC recapitulate all of the above, stress-induced functional abnormalities [103, 105]. Second, either blockade of GC production with the CRF inhibitor, antalarmin, or peripheral action, with the GC receptor inhibitor, mifeprostone (Ru486), prevent emergence of PS and GC-initiated functional abnormalities [104, 105]. A GC-mediated inhibition of synthesis of the three key epidermal lipids that mediate barrier function; i.e., Cer, cholesterol, and FFA, accounts for the negative effects of PS [102, 105]. Accordingly, a

topical mixture of these three lipids largely normalized all of these functions in mice and humans, even in the face of ongoing PS or GC therapy [102, 106].

## Basis for Lipid Abnormalities in AD

**Global Decline in Lipids:** Filaggrin-associated AD is characterized by profound abnormalities in lipid content, distribution, and lamellar membrane organization in lesional skin [107–109], which result in a paracellular barrier abnormality [25, 70, 82]. A moderate impairment of lamellar body secretion, due to a cytoskeletal abnormality, results in entombment of substantial quantities of lamellar bodies within corneocytes, a feature that becomes more prominent once inflammation is established [82]. In addition, KLK signaling of the PAR2 downregulates lamellar body secretion [92], likely providing an additional biochemical signal that accounts for entombment of these organelles in nascent corneocytes. Together, these abnormalities result in incomplete delivery of lamellar body-derived cargo, as well as a paucity of extracellular lamellar bilayers, leading to a global reduction in extracellular lipids [72, 108] (Fig. 6). Finally, not only extracellular lamellar bilayers, but also the covalently-bound lipids that form the corneocyte lipid envelope, decline in AD [110], further contributing to the barrier abnormality.

**Sphingolipid Abnormalities in Atopic Dermatitis:** The most distinctive hallmark of human AD is the repeatedly-noted, selective reduction in the Cer content of affected SC [75, 76]. Several mechanisms likely contribute to the decrease in Cer. As noted above, the barrier-related increase in pH, and a pH-induced increase in KLK activity results in deactivation, and ultimately in accelerated degradation of the Cer-generating enzymes, acidic sphingomylinase and β-glucocerebrosidase (Fig. 5) [74]. Moreover, the cytokine cascade, associated with AD and other inflammatory dermatoses with a barrier abnormality, upregulates production of interferon-γ, which downregulates epidermal synthesis of Cer [111]. Furthermore, noting that neither sphingomyelin nor glucosylceramides accumulate in the SC of AD, Imokawa, et al. (2009) provided evidence that AD epidermis exhibits novel N-deacylation activities that degrade both sphingomyelin and glucosylceramides. However, the genes for these enzymes have not yet been identified in skin; hence, it remains possible that this deacylase activity could be of bacterial origin. Accordingly, several other microbial pathogens that are known to colonize AD elaborate acidic ceramidase activity [112, 113], which could further decrease Cer content. Yet, the sphingoid base content of the SC in AD is *lower* (not higher) than in normal SC [114], arguing against an important role for microbial ceramidases in producing Cer deficiency in AD. Finally, it should be noted that abnormalities in the ratio of sphingoid bases, specifically in sphingosine and sphinganine, could modify lamellar membrane permeability, thereby contributing to the barrier defect in AD [115].

Increased production of the Th2-derived cytokines, IL-4 and IL-13, also further contributes to the decrease in Cer in AD. In experimental animals, IL-4 not only down-regulates serine palmitoyl transferase, the rate-limiting enzyme of ceramide synthesis, but it also blunts the potential benefits of Th1-derived TNF-α on ceramide-generating enzymes [116, 117]. Thus, while Th1 cytokines upregulate Cer production [118], the dominance of Th2 cytokines in AD

likely overwhelms this Th1 response, with profound consequences for epidermal structure and function (Fig. 5).

**Shorter N-Acyl Fatty Acids in AD:** Researchers in Japan and The Netherlands recently reported that the sum of sphingoid bases plus the N-acyl fatty acids (FA) in ceramides declines, in parallel with a decline in the chain length of free and esterified fatty acids (FA) in lesional AD [119–121]. These shorter-chain FA in turn produce abnormalities in lipid organization that likely compromise permeability barrier function in AD [119, 121, 122]. The basis for these chain length abnormalities could prove to be reduced expression of two fatty acid elongases, ELOVL1 and ELOVL4, enzymes required to generate the very long chain N-acyl FA in Cer and FFA in AD [111] (Fig. 5). It is intriguing to speculate that the reduced levels of ELOVs could again prove to be an acquired abnormality due to elevated Th2 cytokines. Alternatively, elevated levels of interferon gamma (IFNγ) downregulate ELOV1 and 4 [69], which could account for reduced N-acyl chain length [111]. Finally, degradation of ELOVs by excessive KLK activity could at least in theory contribute to these abnormalities.

## CONCLUSION: Clinical and Therapeutic Implications

Sustained antigen ingress through a defective barrier leads to a Th2-dominant infiltrate, which then becomes an *additional* cause of inflammation in AD (Fig. 2).While certain antigens, such as cat dander, preferentially trigger childhood AD in FLG-deficient patients [123], the worst offenders are mites and cockroach antigens, which themselves release and activate KLK, resulting in further damage to the barrier [124]. Furthermore, the lipid-depleted barrier in AD may facilitate the penetration of water-soluble haptens, such as nickel. Indeed, nickel-induced, acute allergic dermatitis is more common in humans with FLG-deficient AD than in normals [125126]. Accordingly, correction of the barrier abnormality alone with measures that restore and correct lipid imbalance could prevent and/or ameliorate the barrier abnormality in AD, thereby reducing the inflammatory component in AD [98, 107, 108, 127]. There is now emerging evidence that physiologic lipids, if delivered in sufficient quantities, and at appropriate molar ratios, are effective in the treatment of even moderate-to-severe AD, without any of the safety concerns surrounding glucocorticoids and immunomodulators 108*. Alternatively, those patients with AD due to either single-allele mutations in *FLG*, and/or acquired reductions in FLG, become potential candidates for strategies that either upregulate FLG expression [128129]; or enhance the transdermal delivery of FLG monomers to deficient skin [129]. Yet, it must be noted that the latter approach, though very elegant in theory, may not be practical for a generalized disease such as AD.

## Acknowledgments

This work was supported by NIH grant AR019098, and by the Medical Research Service, Department of Veterans Affairs. These contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIAMS or NIH.

*Conflict of Interest Statement: Dr. Elias is a co-developer of this form of treatment for AD, currently licensed from the University of California to PuraCap Pharmaceutical, LLC. (Dr. Elias is a consultant to PuraCap).

## Abbreviations

| | |
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| **AD** | atopic dermatitis |
| **AMP** | antimicrobial peptides |
| **AR** | amphiregulin |
| **Cer** | ceramides |
| **FLG** | filaggrin |
| **FFA** | free fatty acids |
| **GC** | glucocorticoids |
| **hBD** | human β-defensins |
| **IFNγ** | interferon gamma |
| **IV** | ichthyosis vulgaris |
| **KLK** | kallikreins |
| **LEKTI** | lymphoepithelial Kazal-type trypsin inhibitor |
| **NGF** | nerve growth factor |
| **NS** | Netherton syndrome |
| **PAR2** | protease activator type 2 receptor |
| **PS** | psychological stress |
| **SP** | serine protease |
| **SC** | stratum corneum |
| **TJ** | tight junction |
| **TSLP** | thymic stromal lymphopoietin |

## Glossary

Diverse mechanisms converge on lamellar body secretion, producing the barrier abnormality in atopic dermatitis

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| **Lamellar body** | Small, ovoid, membrane-bound, secretory organelle synthesized by keratinocytes as they reach the stratum spinosum. An intracellular pathway involving the Golgi apparatus produces them. Their lipid molecular contents display a plate like appearance when viewed through high magnification electron microscopy |
| **Ceramides** | A group of amido sphingolipids (ex sphingomyelin and cerebrosides) formed by linking a fatty acid to a sphingoid base ($C_{18}H_{37}NO_2$). |
| **Antimicrobial peptides** | Components of the innate immune system capable of inserting into bacterial phospholipids that kill or slow microbial growth. |

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| **Xenobiotes** | Chemical compounds foreign to living organisms. |
| **Keratohyalin granules** | Substance synthesized by free ribosomes in a keratinocyte as it passes through the epidermis. These granules expand and then interact with tonofilaments to aggregate keratin in corneocytes. |
| **S-100** | Calcium binding proteins that modulate both intra- and extracellular processes in the epidermis, including keratinocyte differentiation. |
| **TSLP** | An IL-7-like cytokine that activates dendritic cells to produce pro-Th2 chemokines and primes naïve T cells to differentiate into Th2 cells. |
| **pH** | A scale measurement of acidity/alkalinity on which a value of 7 represents neutrality. Each unit of change represents a 100-fold change in acidity or alkalinity. Skin pH in a healthy adult ranges from 4.5 to 5.5. Washing skin with soap increases skin pH by approximately 3 units. Skin protease activity is enhanced at pH values above 7.5. |
| **Humidity** | Typically expressed as relative humidity: The amount of water vapor in the air at a specified temperature and pressure relative to the total amount it could hold at those values. |

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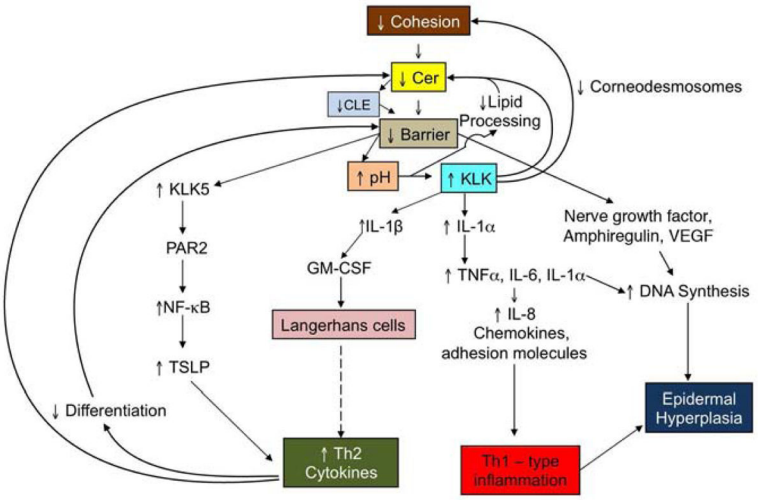


**Fig. 1. Multifunctional Impact of Secreted Lamellar Body Contents**
(Modified from[106])

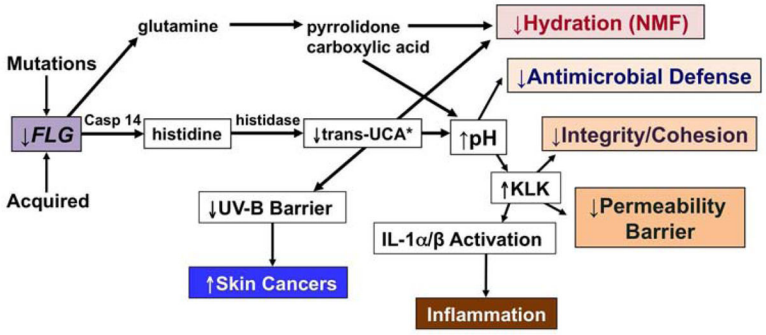


**Fig. 2. Cytokine Cascade Leads To Multiple 'Vicious Cycles' in AD**

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-1, interleukin-1; KLK, kallikrein; NF-κB, nuclear factor kappa B; TNFα, tumor necrosis factor alpha; TSLP, thymic stromal lymphopoietin

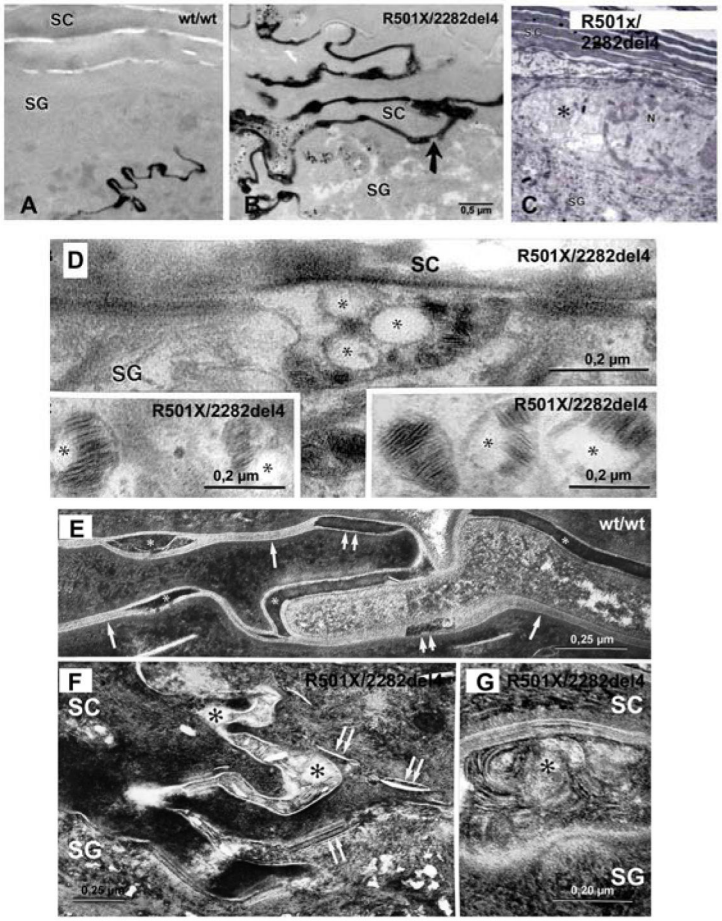


**Fig. 3. Multiple Downstream Consequences of Filaggrin Deficiency in Atopic Dermatitis**

*Trans-urocanic acid (t-UCA) is the most potent endogenous UV-B filter in lightly-pigmented skin. Loss of t-UCA could account for the higher incidence of non-melanoma skin cancers in AD 45. (Modified from Elias & Williams, JID, 2013)

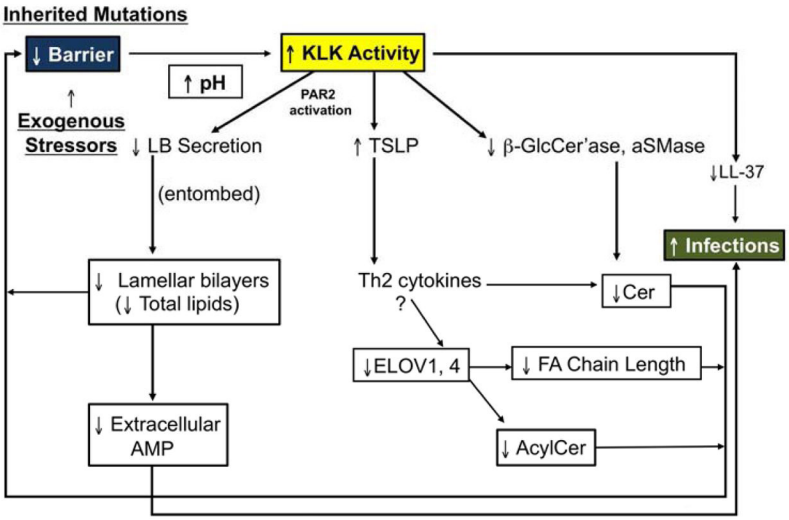


**Fig. 4. Abnormalities That Lead to Paracellular Barrier Abnormality in FLG-Deficient Epidermis**

Lanthanum perfusion in double-allele ichthyosis vulgaris (IV) (**B**) vs. wild-type (**A**) human epidermis. **C**: Retraction of cytoskeleton (asterisks) in double-allele IV. **D** (+ inserts): Impaired loading and secretion of lamellar body cargo in double-allele IV. **F–G**: Post-secretory abnormalities in lamellar bilayer organization and maturation in double-allele IV (arrows). **E:** Normal (wild- type) human epidermis. RuO4 post-fixation (Modified from [25]).

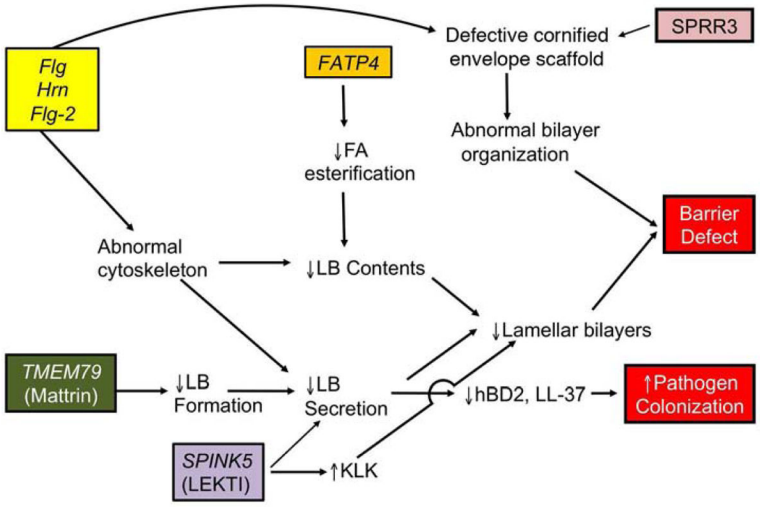


**Fig. 5.**

LESSONS FROM NETHERTON SYNDROME:

Proposed Roles for Increased pH and KLK Activation in Producing Lipid Abnormalities in AD

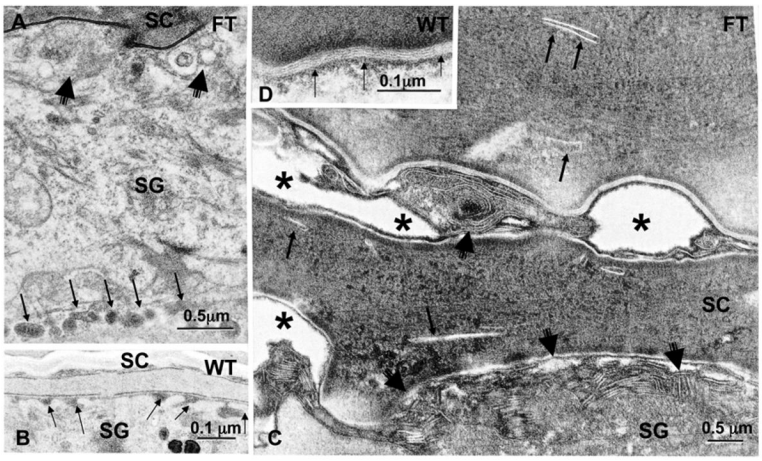


**Fig. 6. How Inherited Abnormalities Converge to Produce Defective Permeability and Antimicrobial Barriers in AD**

Abbreviations: FA, fatty acid; Fatp4, fatty acid transport protein 4; Flg, filaggrin; hBD2, human beta-defensin 2; Hrn, hornerin; LB, lamellar body

## Table 1

Consequences of Inherited Barrier Abnormalities in AD

| Structural Protein | Immediate Structural Consequences | Downstream Changes → Barrier Defect |
|---|---|---|
| **S-100Type** | | |
| ↓Filaggrin | Attenuated CE → Poor Scaffold | Bilayer disorganization |
| | ↑SC pH → ↑KLK | Lamellar body entombment → Lamellar bilayers |
| | ↓SC hydration | ↑TEWL, ↑pro-inflammatory cytokines |
| ↓Hornerin | ? Attenuated CE | ? |
| ↓Filaggrin-2 | ? Attenuated CE | |
| **Other CE Precursors** | | |
| SPRR3 | Attenuated CE → Poor Scaffold | |
| **Enzyme Inhibitor** | | |
| LEKTI 1 (Netherton syndrome) | ↑KLK activity | Destruction of corneodesmosomes, lipid processing enzymes, LL-37 |
| **Lipid Metabolism** | | |
| FATP4 | ↓FFA, FACoA, Esterified FA | ↓Glycerolipids; Detergent effects of excess FFA |
| Mattrin | ↓Lamellar body formation/secretion | ?↓Extracellular lipids |

**Abbreviations**: CE, cornified envelope; FATP4, fatty acid transport protein 4; FFA, free fatty acids; KLK, kallikreins, LEKTI, lympho-epithelial Kazal-type trypsin inhibitor; TEWL, transepidermal water loss

## Table 2

Key Points

| | |
|---|---|
| 1 | A variety of unrelated mutations that compromise epidermal barrier function predispose to the development of atopic dermatitis (AD). |
| 2 | An acquired deficiency in filaggrin also occurs, independent of mutation status. |
| 3 | These mutations converge on the lamellar body secretory system, producing abnormalities in either lamellar body formation, secretion, or post-secretory processing that compromises extracellular lamellar bilayer structure |
| 4 | Theses secretory abnormalities account in large part for the distinctive lipid abnormalities in AD, which included a global decrease in barrier lipids; a further decline in ceramide content; and truncation of the chain lengths of free and esterified fatty acids. |
| 5 | The same pathogenic sequence compromising antimicrobial defense accounts at least in part for colonization by *S. aureus* and other pathogens in AD. |
| 6 | Based upon the above, rational therapy should address and correct filaggrin status, and/or the lipid abnormalities in AD. |