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Cutaneous Barrier Dysfunction in Allergic Diseases

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Abstract

The fundamental defect(s) that drives atopic dermatitis (AD) remains controversial. “Outside in” proponents point to the important association of filaggrin gene mutations and other skin barrier defects with AD. The “inside out” proponents derive support from evidence that AD occurs in genetic animal models with overexpression of Type 2 immune pathways in their skin, and humans with gain-of-function mutations in their Type 2 response develop severe AD. The observation that therapeutic biologics, targeting Type 2 immune responses, can reverse AD provides compelling support for the importance of “inside out” mechanisms of AD. In this review, we propose a central role for epithelial cell dysfunction that accounts for the dual role of skin barrier defects and immune pathway activation in AD. The complexity of AD has its roots in the dysfunction of the epithelial barrier that allows the penetration of allergens, irritants, and microbes into a cutaneous milieu which facilitates the induction of Type 2 immune responses. The AD phenotypes and endotypes that result in chronic skin inflammation and barrier dysfunction are modified by genes, innate/adaptive immune responses, and different environmental factors that cause skin barrier dysfunction. There is also compelling evidence that skin barrier dysfunction can alter the course of childhood asthma, food allergy, and allergic rhinosinusitis. Effective management of AD requires a multi-pronged approach, not only restoring cutaneous barrier function, microbial flora, and immune homeostasis, but also enhancement of skin epithelial differentiation.

Keywords

atopic dermatitis; food allergy; skin barrier; peanut allergy; epithelial barrier

INTRODUCTION

Allergic diseases such as atopic dermatitis (AD), food allergy (FA), and asthma affect more than 30% of the general population (1–3). These diseases have significant health and socio-economic effects. Allergic diseases are well documented to be associated with epithelial barrier dysfunction, allowing tissue penetration of allergens, irritants, and microbes. These

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then lead to the release of epithelial cytokines such as thymic stromal lymphopoietin (TSLP) and IL-33, which play a pivotal role in driving Type 2 immune and inflammatory responses (4,5). Although cytokines such as interferon gamma, IL-17, and IL-22 can modify the course of allergic responses, Type 2 cytokines such as IL-4, IL-13, TSLP, and IL-33 play a central role in the development of allergic diseases (6,7). AD, in particular, is the most atopic of all allergic diseases as environmental antigens and foods, processed in the skin elicit an IgE response. Childhood onset of AD is often associated with FA, asthma, and allergic rhinitis, i.e. the atopic march (8). The skin barrier dysfunction in AD is thought to play a key role in the atopic march. It starts with AD, followed by epicutaneous allergen sensitization and FA. The link from AD to respiratory allergy is more controversial; however, progression of the atopic march is facilitated in patients who develop IgE to foods and inhalant allergens (9), and is strongly associated with filaggrin loss-of-function gene mutations. Childhood asthma onset-specific gene loci have identified the skin as an important target tissue (10). Severity, age of onset, and duration of AD are risk factors for the atopic march (11,12).

In this review, we will discuss the role of cutaneous barrier dysfunction in the pathogenesis of AD, particularly the relationship of AD with FA. The relative importance of these individual abnormalities can be teased out using animal models and therapeutic interventions; however, in most patients, the progression of AD requires both the skin barrier defect and immune pathway activation. For a discussion of the contributions of an excessive Type 2 innate and acquired immune response in AD, the reader is referred to a recent review by Honda and Kabashima (13). We propose that the dualistic role of skin barrier and immune abnormalities has its roots in the lack of terminal keratinocyte differentiation in the skin and propose that durable therapeutic approaches require combination therapy to target both the defective skin barrier and Type 2 immune activation in AD. This has important clinical implications for new approaches to controlling AD and progression of the atopic march.

CUTANEOUS BARRIER DYSFUNCTION IN AD

Skin barrier dysfunction is the hallmark of AD. A strong skin barrier in healthy individuals is needed to repel the invasion of microbes, allergens, and irritants from the environment, thereby preventing engagement of the Type 2 enhancing immune pathway that occurs in the skin. In a birth cohort study, increased transepidermal water loss (TEWL) at day 2 of life, occurred one year prior to the onset of AD and FA (14, 15). Since increased TEWL is a biomarker of skin barrier dysfunction, this study suggests early intervention is required to prevent epicutaneous allergen sensitization. Increased TSLP has also been found in the skin prior to onset of AD suggesting that prevention of the atopic march also requires intervention with Type 2 pathways (16).

Filaggrin deficiency as the paradigm for skin barrier dysfunction (Figure 1)

Loss-of-function filaggrin (*FLG)* gene mutations are the strongest known genetic risk factor for AD (17). The presence of *FLG* mutations increases AD risk more than three-fold compared to the general population. It is also a disease modifier predisposing to earlier AD onset, persistence of AD, and increased disease severity. There are ethnic differences in the

types of *FLG* mutations found in AD. *FLG* mutations are particularly common in northern Europeans with R501X and 2282del4 as the major *FLG* mutations. In Asian populations, *FLG* P478S and C3321delA variants, not commonly found in European populations, are associated with increased risk of AD (18). In African American children, loss-of-function mutations in *FLG*2 are associated with increased AD risk. Several novel *FLG* gene mutations, not commonly seen in European Americans, were recently described in African American AD.

Mouse models have demonstrated that *FLG* gene mutations are associated with enhanced percutaneous microbial and allergen penetration and reduced inflammatory thresholds to irritants and haptens (19,20). Knockdown of *FLG* expression has been shown to impair keratinocyte differentiation of human keratinocyte organotypic cultures (21). Epithelial damage leads to innate immune activation, including release of pro-inflammatory cytokines and chemokines by keratinocytes (22), and enhanced antigen presentation by Langerhans cells and dermal dendritic cells (23). Reduced levels of acidic filaggrin breakdown products raise skin pH and activate skin proteases (24–26), thereby contributing to skin barrier dysfunction. Protease activated receptor activation has also been shown to induce the pro-Th2 cytokine, TSLP (27).

Beyond *FLG* null mutations, a number of additional factors play an important role in the regulation of filaggrin expression and the skin barrier in general. For example, Type 2-mediated cutaneous inflammation can result in reduced filaggrin expression in AD skin, even in subjects without *FLG* mutations (28). The combination of Type 2 cytokines with heterozygote *FLG* gene mutations can profoundly reduce *FLG* to almost undetectable levels. Reduced *FLG* intragenic copy number is an independent risk factor of AD (29). DNA methylation of the CpG site in *FLG* gene region has also been reported to significantly increase AD risk (30). The *FLG* gene is only one of approximately 45 genes within the epidermal differentiation complex (EDC) on chromosome 1q21. Many of these genes, including involucrin (IVL) and loricrin (LOR), may also contribute to AD cutaneous barrier dysfunction. The levels of hornerin and other filaggrin-like proteins, including *FLG*2, are also decreased in the skin of AD patients (31,32), but filaggrin deficiency appears to have the greatest impact on skin barrier structure and function in AD. IL-22 has also been shown to be overexpressed in the skin of severe AD, and it inhibits filaggrin skin expression (33); however, in contrast to Type 2 cytokines, it does not inhibit keratinocyte antimicrobial peptide production (34). Other proinflammatory cytokines, like TNFa (35), IL-25, or IL-33 (36), have also been found to inhibit filaggrin expression.

Failure of terminal keratinocyte differentiation in AD skin (Figure 2)

AD epidermis is characterized by broad defects in terminal keratinocyte differentiation (37). This allows enhanced allergen penetration through the epidermis and systemic IgE sensitization. There is an expansion of cells in the stratum basale (SB) layer, with a concomitant reduction in cells of the stratum spinosum and stratum granulosum layers (38). Hyperproliferating epithelium is associated with overexpression of KRT6/KRT16 (38). Consistent with a block in terminal keratinocyte differentiation, AD skin has reduced

expression of mature skin barrier proteins including filaggrin, involucrin, Loricrin, antimicrobial peptides, and beta defensins (28,35,40).

Tight junctions in the granular layer form an additional component of the skin barrier, limiting penetration of allergens and pathogenic microbes, facilitating paracellular passage of soluble mediators, and regulating TEWL. Tight junctions are composed of transmembrane proteins such as claudin-1 (CLDN1), which are essential for skin barrier function and control of TEWL regulation (41). CLDN1 is reduced in the skin of AD patients (42). Polymorphisms in the CLDN1 gene have been found in AD, particularly those with a history of eczema herpeticum (43). Knockdown of CLDN1 expression in keratinocytes enhances HSV-1 infectivity.

S100A7, S100A8, and S100S9 proteins are upregulated in AD skin (44, 45). These proteins act as amplifiers of the immune response. As an example, S100A9 activated keratinocytes causes a selective increase in IL-33 production. Th2 cytokines inhibit S100A11 protein expression, which is required for the regulation of skin barrier integrity (46).

Disruption of Keratinocyte Differentiation in AD skin

Evolving research suggests that keratinocyte differentiation is disrupted in AD with hyperproliferation of stem cells in the basal layer (Figure 3). Cutaneous Notch signaling plays a key role in the promotion and maintenance of the keratinocyte differentiated state (47,48), while Wnt activity is essential for stem cell maintenance and supports cell proliferation (49). Inhibition of Notch receptor expression has been shown in AD skin (50,51), while in healthy control patients significant Notch expression was observed in the suprabasal epidermal layers (50). Reduced Notch expression appears to be a selective feature of AD skin, as increased epidermal Notch expression has been found in other inflammatory skin diseases such as psoriasis and lichen planus (52,50).

Dry skin in AD with increased TEWL has been linked to increased aquaporin 3 (AQP3) expression (53,54). Increased AQP3 expression has been found in the stratum basale and stratum spinosum of patients with AD (54). Interestingly, AQP3 has been identified as a transcriptional target of Notch1. Inhibition of Notch signaling increased the expression of AQP3 (55). Thus, decreased Notch signaling in AD may increase AQP3-mediated TEWL leading to dry skin.

Epidermal Notch deficiency in mice induces AD-like skin pathology, with dry skin, acanthosis, spongiosis, hyperkeratosis, and massive dermal infiltration of eosinophils and mast cells (50,56,51,57). Notably, epidermal Notch deficiency is associated with a significant production of TSLP by keratinocytes, with increased numbers of DCs, enhanced expression of IL-4 and IL-13, and increased serum IgE levels (50,56,51,57). Notch deficient murine keratinocytes develop pronounced defects of epidermal barrier integrity and cornified envelope formation (58,56). Keratinocyte-derived TSLP has recently been shown to stimulate cutaneous sensory neurons to promote itch (59). Thus, there may be a direct link between epidermal Notch deficiency and TSLP-induced pruritus in AD.

We propose that Notch deficiency in AD skin is fundamental to the inhibition of epidermal differentiation and skin barrier deficiency. At the same time, Notch insufficiency in the epidermis results in keratinocyte hyperplasia, as cells in the basal layer expand, while maintaining their proliferative, undifferentiated state, thus, reprogramming epidermal differentiation in AD skin. Notch activation is known to inhibit the Wnt pathway (60,61). Reduction in signal strength induced by loss of Notch1 in epithelial cells augments Wnt signaling, and induces a permissive environment for the outgrowth of proliferating cells in murine models (62,63). Evidence for Wnt/beta-catenin pathway activation has been shown in allergic rhinosinusitis (64,65) and psoriasis (66,67), supporting epidermal hyperplasia. Lastly, as stated above, Notch deficiency supports type 2 inflammation.

At the same time, skin scratching, environmental insults and bacterial colonization in AD promote release of alarmins (IL-1alpha, IL-33, IL-18, IL-36), that also promote epidermal hyperplasia and establishment of type 2 inflammation in the skin (68,69). It is noteworthy that epidermal hyperplasia would be expected to prevent cells from terminal differentiation. Of interest, IL-1 family member cytokines have been shown to activate Wnt expression in keratinocytes (66) thus adding to the complexity of inflammatory events in AD.

Microbial Dysbiosis reflects Cutaneous Barrier Dysfunction in AD

AD skin is predisposed to colonization or infection by pathogenic microbes, most notably *Staphylococcus aureus* (70,71). Work in our laboratory and others have demonstrated that IL-4 and IL-13 promote *S. aureus* invasion/colonization (40,72–74) due to inhibition of epidermal barrier function (75), induction of *S. aureus* skin binding sites (e.g. fibronectin), and decreased antimicrobial peptide production in AD skin (40,76,77). The pivotal role of IL-4 and IL-13 in causing *S. aureus* colonization was recently demonstrated by a report that dupilumab, a therapeutic monoclonal antibody against IL-4 receptor alpha, an antagonist of IL-4 and IL-13 signaling, caused greater reduction in *S. aureus* colonization than placebo in AD patients (78). Superantigen-producing *S. aureus* skin colonization has been shown to be potent activators of IL-4, IL-13 and IL-22 production in AD (79), supporting the role of *S. aureus* in inducing and maintaining AD skin inflammation.

Longitudinal studies have demonstrated that *S. aureus* colonization emerges during the exacerbation of AD (80,81). Recent studies support a bidirectional dialogue between skin bacteria and host keratinocytes, with commensal microbiota educating host immune responses and, conversely, atopic immune signaling shaping microbial dysbiosis (80,82).

Next-generation sequencing of bacterial DNA collected from AD skin has documented increased *S. aureus* colonization and decreased bacterial diversity (81,83). Specific *S. aureus* strains have been associated with AD severity (84). *S. aureus* clones identified in severe AD patients were enriched for the expression of virulence factors. Murine skin colonization models have demonstrated *S. aureus* strain-specific differences in elicitation of skin inflammation and immune signatures characteristic of AD patients. Specifically, *S. aureus* isolates from AD patients with more severe flares induced epidermal thickening and expansion of cutaneous Th2 and Th17 cells, suggesting that functional differences of staphylococcal strains may contribute to the complexity of AD disease (84).

Normal skin microbiota play an important role in the development of innate barrier immunity (85–87), limit of pathogen invasion (88), and control of T regulatory cell function (89). Birth cohort studies have shown that the presence of coagulase-negative *Staphylococcus* spp. at two months of life might protect infants against later development of AD (90). Early-life skin colonization with *S. aureus* may also contribute to AD onset in infancy (91). Several virulence factors (including lytic toxins, enterotoxins, proteases, etc.) produced by *S. aureus* contribute to AD by acting on keratinocytes (cell lysis, proinflammatory cytokine production, inhibition of keratinocyte differentiation program) and immune activation (T cell clonal expansion, production of proinflammatory cytokines) (75,92,93). *S. aureus* exploits epidermal barrier defects in AD to trigger cytokine expression (94). Activation of serine proteases is essential for *S. aureus* penetration into the skin (95). Importantly, antimicrobial function of commensal microflora is critical for controlling *S. aureus* colonization (88).

Cytokine Role in Barrier Dysfunction

Overproduction of IL-4/IL-13 creates a permissive environment for *S. aureus* growth and attachment to AD skin (96). In addition, AD skin has reduced filaggrin expression, accompanied by reduced levels of FLG breakdown products (PCA, UCA) on the skin. Acidification of the skin by filaggrin breakdown products has been shown to reduce expression of *S. aureus* secreted and cell wall-associated proteins, including proteins involved in colonization (clumping factor B, fibronectin binding protein A) and immune evasion (protein A) (97). In addition, filaggrin breakdown products inhibit the expression of iron-regulated surface determinant A by *S. aureus*. In contrast, reduced levels of filaggrin breakdown products support *S. aureus* colonization. Th2 cytokines can also enhance the effects of staphylococcal products. For instance, keratinocytes in AD, as compared to normal skin, have increased sensitivity to alpha-toxin, a cytolytic toxin produced by *S. aureus*. Differentiated keratinocytes are protected from cell death, whereas cells treated with IL-4/IL-13 have increased sensitivity to alpha toxin-induced lethality (72). The combination of IL-4/IL-13 induce biochemical changes that decrease levels of acid SMase, an enzyme that cleaves an alpha toxin ligand called sphingomyelin (75). SMase and its enzymatic product, phosphocholine, prevent IL-4/IL-13-mediated increases in alpha toxin-induced cell death (75).

We recently demonstrated an interplay between *S. aureus* cell wall component lipoteichoic acid (LTA) and IL-4/IL-13 in the inhibition of wound healing in AD skin (98). We found that keratinocytes are highly responsive to LTA (with change in expression of genes involved in regulation of epidermal development), wound responses, keratinocyte proliferation, regulation of cell differentiation, and Notch signaling pathways (99). We have reported that staphylococcal LTA inhibits the expression of the early keratinocyte differentiation markers, keratins (KRT1, KRT10), desmocollin (DSC1), and desmoglein (DSG1), that are essential for skin barrier function and have determined that these events are p63 dependent. Interestingly *S. aureus* colonization has also been associated with onset of food allergy (100). This may reflect the detrimental effect of *S. aureus* on the skin barrier and enhanced epicutaneous food allergy sensitization.

Lipid Abnormalities (Figure 4)

Dr. Christian Cole and colleagues stratified the analysis of AD skin transcriptome based on *FLG* gene mutations and found that AD with normal *FLG* genotype have aberrant changes in the expression of enzymes involved in the metabolism and synthesis of lipids (101). These observations suggest the importance of lipid metabolism in AD independent of *FLG* genotype. Extracellular lipids account for almost 10% of the stratum corneum (SC) structural mass (102–105). Skin lipids can also have anti-inflammatory and antimicrobial properties (106). Free fatty acids and sphingoid bases have documented antimicrobial activity, including activity against *S. aureus* (107,108). Several free fatty acids serve as natural agonists for peroxisome proliferator-activated receptor transcriptional factors that are essential for the regulation of lipid metabolism enzymes in the skin (109).

Several research groups have demonstrated reduction in skin ceramide levels, in particular in esterified ω-hydroxy fatty acid (EO) sphingosine (S) ceramides (EOS-Ceramides), in AD skin in parallel with a decline in free fatty acid chain length (110,111). These changes in skin lipid composition resulted in abnormal lipid organization and increased TEWL in AD skin. Notably, changes in ceramide levels and free fatty acids chain length distribution did not correlate with FLG genotype, but correlated with AD severity and levels of FLG breakdown products.

Concurrent reduction in ceramide and free fatty acid chain length in the SC of AD patients suggested alterations in the common elongase synthetic pathway for ceramides and free fatty acids. We investigated whether the hyperactivated Type 2 immune response altered AD skin lipid metabolism. To address this question, we analyzed SC lipids from AD subjects and IL-13 skin-specific transgenic mice (112). Mass spectrometric analysis of lesional SC from AD subjects and IL-13 transgenic mice revealed an increased proportion of short-chain (N-14:0–24:0) non-hydroxy fatty acid sphingosine ceramides (NS-ceramides) and 14:0–22:0-lysophosphatidylcholines (LPC) with a simultaneous decline in the proportion of corresponding long-chain species (N-26:0–32:0 sphingolipids and 24:0–30:0-LPC) when compared to healthy controls. An increase in short-chain LPC species was also observed in non-lesional AD skin. Similar changes were observed in IL-4/IL-13-driven responses in Ca^{2+}-differentiated human keratinocytes in vitro, all being blocked by STAT6 silencing with siRNA, a master transcriptional factor regulator of IL-4/IL-13 signaling. RNA sequencing analysis performed on SC of AD as compared to healthy subjects identified decreased expression of the fatty acid elongases, ELOVL3 and ELOVL6, that accounted for the observed changes in AD skin lipids. IL-4/IL-13 also inhibited ELOVL3 and ELOVL6 expression in keratinocyte cultures in a STAT6-dependent manner. Downregulation of ELOVL3/ELOVL6 expression in keratinocytes by siRNA decreased the proportion of long-chain fatty acids globally and in sphingolipids. Thus, our data strongly support the pathogenic role of Type 2 immune activation in AD skin lipid expression (112).

Environmental Factors

Environmental or climatic factors can also adversely impact skin barrier integrity, thereby contributing to AD risk and severity. It has also been documented that mechanical damage,

including repetitive scratching, use of detergents, humidity, exogenous proteases, and air pollution, have a negative impact on FLG expression (113).

Cross-talk between the skin and gut has recently been demonstrated in a mouse model of AD and FA. In these experiments, tape stripping mouse skin, used as a surrogate for scratching or mechanical injury, caused expansion and activation of small intestinal mast cells (MCs), increased intestinal permeability, and promoted food anaphylaxis in mouse models (114). The mechanism involved release of IL-33 systemically from keratinocytes after skin tape stripping. IL33 acted on intestinal tuft cells to secrete IL-25 to drive the expansion and activation of intestinal innate lymphoid cells (ILC2s), which provided IL-4 and IL-13 that targeted MCs to expand in the intestine. Mice with skin induced MC expansion had increased IgE-mediated food anaphylaxis in this study. Duodenal mast cells were also increased in biopsies from patients with AD and FA. These studies suggest that, in addition to promoting cutaneous sensitization to food antigens, scratching may play an important role in IgE-mediated food anaphylaxis in AD by expanding and activating intestinal MCs.

CAN EPITHELIAL PROFILING BE USED TO DECIPHER AD PHENOTYPES?

The development of precision medicine approaches to AD require us to develop noninvasive methods to better understand mechanisms underlying the various clinical phenotypes of AD (115). Although AD is often referred to with standard clinical definitions which include pruritus and certain patterns of skin inflammation, this “one-size-fits-all” approach fails to accommodate the complexity of this skin disease. AD patients often present with characteristic phenotypes that transcend local patterns of skin inflammation. These include associations with food allergy and the atopic march, propensity to *S. aureus* colonization or infection, disseminated viral infection (e.g. eczema herpeticum), different ages of AD onset (e.g. early vs late onset of AD), non-responders to therapy (including corticosteroid insensitivity or dupilumab non-responders), and AD severity (mild vs severe courses of illness). Our current fund of knowledge falls short of accounting for these various AD phenotypes, but evolving advances in technology facilitated by a multi-omics approach are expected to provide new insights that explain these various phenotypes and endotypes.

The strong association of *FLG* null mutations with AD, FA, and childhood associated asthma suggests skin barrier dysfunction contributes to the atopic march (8). Clinically, this is supported by reports that risk factors for the development of peanut allergy in children include the epicutaneous application of peanut-containing creams or oils, skin infection, and severity of AD (116). Importantly, several clinical studies support the concept that children become allergic to peanut through environmental exposure (117,118). Furthermore, household peanut consumption was reported to highly correlate with peanut levels in house dust (118).

Only one third of AD patients, however, develop FA (119). As part of a NIH/NIAID Atopic Dermatitis Research Network (ADRN) protocol, we used a minimally invasive skin tape strip (STS) measure of the SC in combination with a comprehensive multi-omics approach to determine whether the non-lesional skin of AD with FA (AD+FA+) children have

superficial skin biomarkers which distinguish them from AD without FA (AD+FA-) and non-atopic (NA) children (120). Despite similar skin disease severity, the SC integrity and filaggrin content were significantly lower in children who were AD+FA+ as compared to AD+FA-. Lipid profiling of the SC in the AD+FA+ group revealed a relative reduction in the ultra long chained ceramides, esterified ω-hydroxy fatty acid (EO) sphingosine (S) ceramides (CER) required for normal skin barrier function. At the same time, a significant increase in NS CER levels was observed in AD+FA+ skin samples, resulting in a disproportionate decrease in EOS CER in the skin of these patients. Shotgun metagenomic studies revealed that the skin of AD+FA+ children was colonized with an overabundance of *S. aureus*. Interestingly, STS proteomics revealed an immature keratin profile consistent with keratinocyte hyperproliferation in the SC of AD+FA+ participants. A network analysis demonstrated keratin 5, 14 and 16 expression, and reduced filaggrin breakdown products, were strongly correlated with AD+FA+. Along with increased TEWL, these were the most important predictors of AD+FA+.

Consistent with the skin barrier dysfunction in non-lesional skin, AD+FA+ individuals had high dendritic cell and Type 2 immune activation profiles in their skin tape transcriptome. Interestingly, transcripts for the Type 2 cytokines IL13, CCL17, and CCL22 were elevated in both AD+FA+ and AD+FA- compared to NA. However, transcripts for Type 2 immune receptors, i.e. IL4R, CCR8, and CRLF2 (TSLP receptor), were higher in AD+FA+ as compared to AD+FA- or NA. These data suggest that the nonlesional skin of AD+FA+ children exhibit a unique constellation of skin biomarkers that distinguish them from AD +FA- and NA children. These data support the importance of skin barrier dysfunction in the pathogenesis of epicutaneous sensitization to environmental foods and may contribute to the persistence or severity of FA by chronically stimulating Type 2 immune responses in the skin.

CLINICAL IMPLICATIONS

The finding that cutaneous barrier dysfunction occurs in the non-lesional skin of children with AD, FA, and possibly childhood asthma, has important clinical implications (121). This concept suggests the need to institute early skin care even before the onset of clinical eczema and the potential importance of doing total body skin care beyond the inflammatory skin rash observed in AD (122). A multipronged approach for the prevention and management of atopic infants and children should be considered and are summarized in Figure 5.

Prevention

Several, but not all, birth cohort studies have reported that the use of emollients to improve the skin barrier can prevent occurrence of eczema (123–125). In the successful studies, skin emollients reduced the occurrence of AD by approximately 50%. The reason for lack of uniform success after application of skin emollients requires further study, but it is important to note that not all emollients have the same effect on the skin and it has been proposed a skin emollient that reflects the lipid composition of the skin is needed in order to obtain

maximum benefits (126). Other factors should also be considered, including time of introduction and adherence to therapy.

Proactive Therapy

The concomitant often subclinical allergic inflammation that accompanies atopic skin can also reduce skin barrier function and may drive IgE responses to occur after foods have penetrated the skin. Duration and severity of eczema is associated with occurrence of FA (116). Therefore, prevention of FA may require proactive skin barrier and anti-inflammatory therapy to reduce Type 2 immune responses to epicutaneous allergen sensitization. In this regard, a recent report found that early aggressive treatment with moisteurizer and topical corticosteroids shortened the duration of eczema in infants and resulted in fewer food allergies at 2 years of age (127). Antagonism of Type 2 cytokines such as IL-4, IL-13, TSLP, or IL-33 may enhance epithelial function and reduce allergen sensitization in patients with established AD (128).

Environmental Control

Since low humidity, skin irritants (including detergents, pollutants, and hard water), and environmental allergens (like dust mite derived serine proteases) are known to induce eczema, avoidance of these environmental factors may prevent cutaneous barrier dysfunction. Studies report that household consumption of food allergens and exposure to environmental foods in household dust result in potential epicutaneous sensitization (129,130). The information from these studies lead to questions about how to change or control these environmental factors, such as if household members should restrict their food ingestion around food allergic children or if a skin barrier cream should be used to prevent epicutaneous sensitization. Exploring these questions will lead to understanding what methods work best and what variety of methods may be available to patients and their families.

Microbiome

S. aureus is known to breakdown the skin barrier and inhibit T regulatory cell activity while *S. aureus* colonization has been found to predate or occur during the development of AD and FA (70,71). Recent studies suggest *S. aureus* colonization is increased on AD skin due to loss of commensal bacteria that produce antimicrobial peptides (88). Studies in the Atopic Dermatitis Research Network are examining whether targeted transplantation of *S. hominis* can reduce *S. aureus* colonization in AD (NCT03151148). An additional clinical study is evaluating whether topical application of the Gram-negative coccobacillus, *Roseomonas mucosa,* can be used to treat AD (NCT03151148). Another approach is to determine whether swabbing neonates born by caesarian section with mother's vaginal microbiota will reduce allergen sensitization (NCT03567707).

Alternative Considerations

Vitamin D is critical in regulating skin development, including formation of filaggrin, lipid lamellae formation, and induction of innate immune responses (131). Therefore maintaining the normal vitamin D levels is essential for the generation and maintenance of the skin

barrier. Importantly, direct sunlight has been found to be even more beneficial in reducing the incidence of infant eczema than vitamin D supplementation (132). Other treatments that are under development include experimental filaggrin replacement therapies, drugs that skip over loss of function FLG mutations, and topical FLG monomers (133). A comprehensive summary of the approved therapeutics and pharmacological therapies in development for the treatment of AD was provided in a recent review (6).

CONCLUSIONS

The clinical manifestations and skin pathology in AD are driven by cutaneous barrier dysfunction and an overly active Type 2 immune response which is exaggerated by skin injury from environmental factors including scratching, *S. aureus* colonization, allergen penetration, stress, irritation from pollution, detergents, hard water, etc. Causes of this abnormal skin barrier are complex and driven by a combination of genetic, environmental, and immunologic factors which affect gene expression, structural proteins, and lipid profiles. These factors likely account for the heterogeneity of AD onset, severity, and natural history of this skin disease. *FLG* gene mutations are the most profound single-gene defects identified in AD, but the most cause common of filaggrin deficiency stems from skin immune activation and environmental factors. Filaggrin deficiency promotes skin inflammation and inflammatory cell infiltration due to increased percutaneous transfer of antigens and chemical haptens, alteration of skin acidification. This in turn supports the activation of skin proteases which further inhibits skin barrier homeostasis and also supports the onset of Type 2 inflammatory response through TSLP and IL-33 activation.

Skin-gut interactions have now been demonstrated and have their origins in skin injury induced release of IL33 from keratinocytes leading to intestinal mast cell hyperplasia (114). Importantly, systemic release of TSLP may contribute to high circulating IgE levels which promote not only FA, but respiratory allergy (12). AD pathobiology evolves from a complex interaction of epidermal barrier disruption, high Type 2 immune response, and an imbalanced skin microbiota which promotes cutaneous barrier dysfunction. The development of noninvasive skin sampling techniques will allow early identification of abnormalities in the skin barrier and facilitate early intervention. These new approaches will play a key role in targeting high risk patient populations for early introduction of cutaneous epithelial repair and therapies which prevent progression of Type 2 immune activation. We believe that the interplay between genetic predisposition, microbial colonization, and Type 2 inflammatory responses are important for the development of epidermal barrier abnormalities and onset of allergic responses. Furthermore, issues of adherence to management programs, timing of intervention, and duration of skin barrier repair are unknown. Beyond prevention is the challenge of effective intervention in a vulnerable population. Onset of lesional AD requires effective control of local and systemic immune activation for optimal management. Early intervention (microbiome, biologics targeting Type 2 immune activation) may improve long term outcomes for AD and reduce the systemic allergen sensitization leading to associated allergic diseases in the gastrointestinal and respiratory tract. An important finding is that AD skin is associated with a hyperproliferative epidermal compartment accompanied by reduced terminal keratinocyte differentiation independent of filaggrin mutations and immune activation. The key challenge

now is identification of interventions directed towards the protection of skin barrier function in early infancy to prevent onset of Type 2 inflammatory responses and development of allergy. Accessibility of AD skin tissue to repeated mechanistic sampling is likely to provide a window to our fundamental understanding of allergic disease.

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Abbreviations used

AD	atopic dermatitis
CER	ceramide
CLDN1	claudin-1
EDC	epidermal differentiation complex
ELOVL	fatty acid elongase
FA	food allergy
EOS	ω-hydroxy fatty acid (EO) sphingosine (S)
FLG	filaggrin
IL	interleukin
IVL	involucrin
KLK	kallikrein proteases
KRT	keratin
LOR	loricrin
LTA	lipoteichoic acid
NMF	natural moisturizing factor
NS	ceramides (non-hydroxy fatty acid sphingosine ceramides)
PCA	pyrrolidone carboxylic acid
TEWL	transepidermal water loss
TGM1	transglutaminase 1

SEB	staphylococcal enterotoxin B
SB	stratum basale
SC	stratum corneum
SG	stratum granulosum
SMase	sphingomyelinase
SPRR	small proline rich protein
SS	stratum spinosum
STAT	signal transducer and activator of transcription
TSLP	thymic stromal lymphopoietin
UCA	urocanic acid
ULCFA	ultra long chain fatty acid

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"What Do We Know?"

- Filaggrin is abnormally low in the skin of peanut allergic subjects irrespective of concomitant AD. Low filaggrin results from genetic mutations, environmental factors and immune activation.
- The lack of terminal keratinocyte differentiation contributes to cutaneous barrier dysfunction in AD.
- Nonlesional skin in AD with FA have features of cutaneous barrier dysfunction that are as abnormal as lesional AD.
- Cutaneous barrier dysfunction allows allergens unrestricted entry into the skin to activate release of IL33 and TSLP from keratinocytes and induction of Type 2 immune responses.
- *S. aureus* colonization in AD is the result of excessive Type 2 inflammation and reduction in beneficial commensal bacteria.

"What is Still Unknown?"

- What is the time frame for prevention of AD?
- What is the ideal emollient for skin barrier protection?
- Do skin emollients have the same effect on non-atopic versus atopic skin?
- What are the causes of different AD phenotypes? Can they be distinguished by epithelial profiling?
- What predictive tests will inform us for precision medicine approaches in AD?

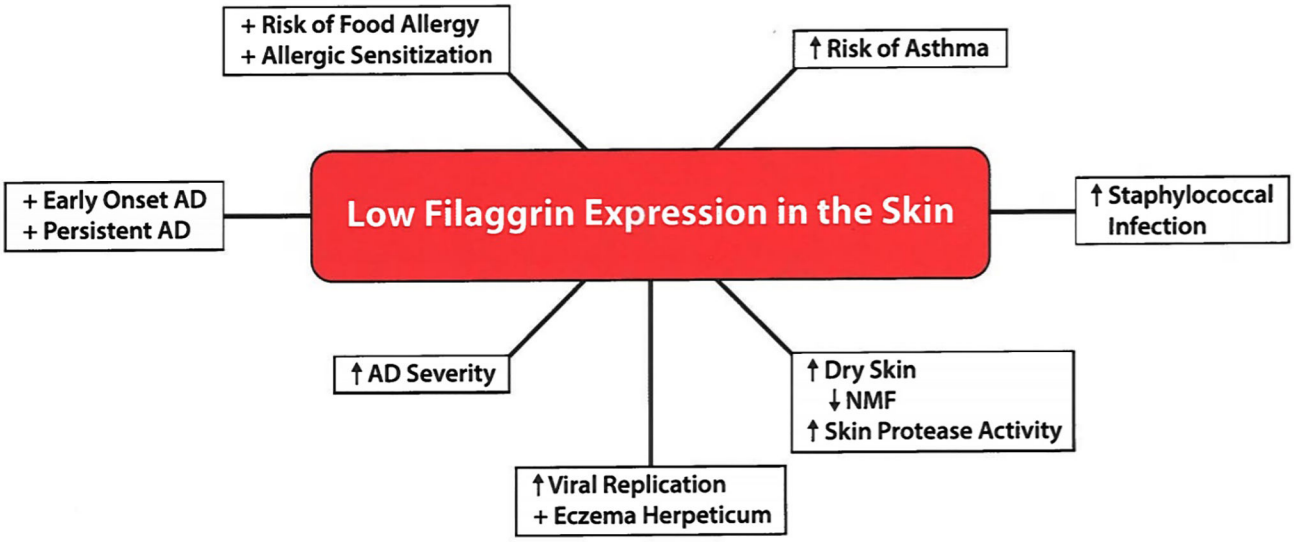
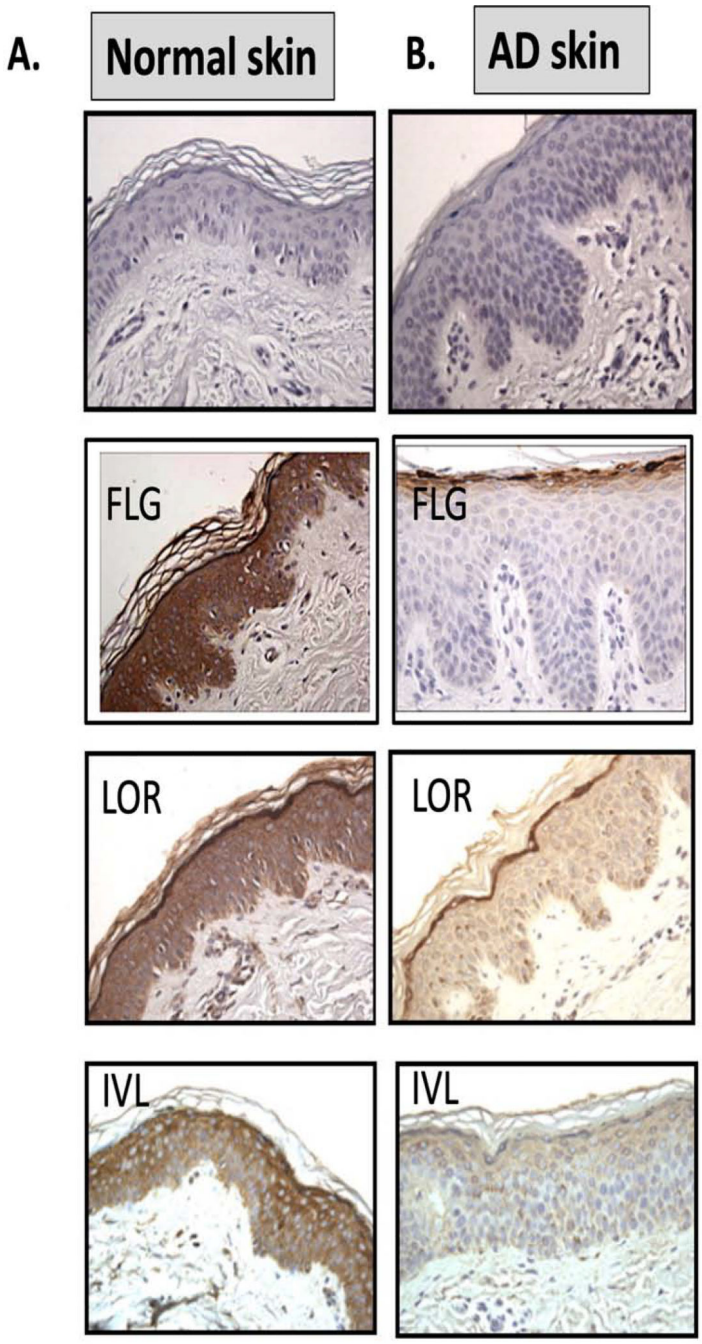


Figure 1.
Consequences of Filaggrin Deficiency on Atopic Dermatitis and Modifying the Course of Allergic Diseases.



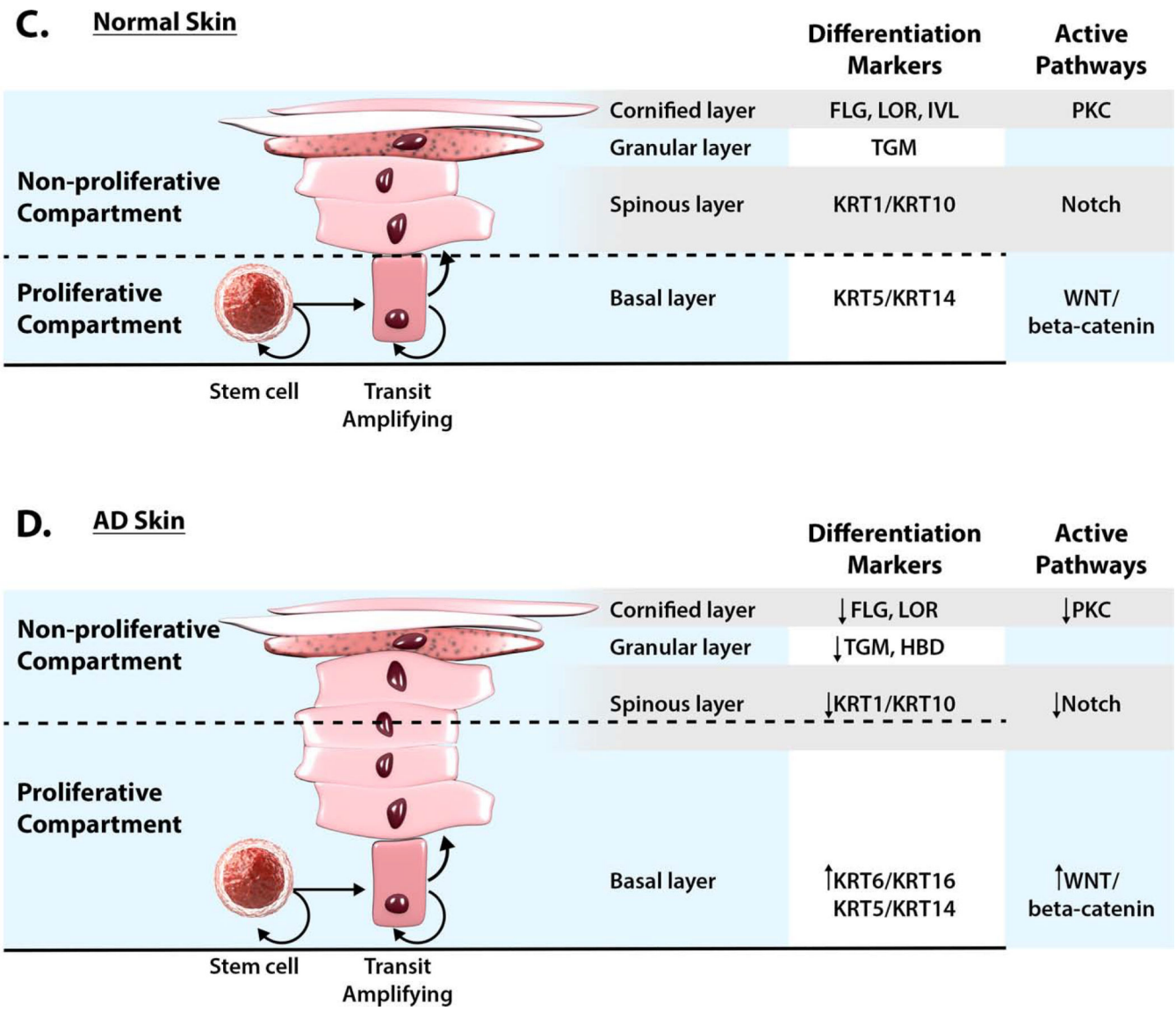


Figure 2. Lack of Keratinocyte Terminal Differentiation in Atopic Dermatitis.

Abnormalities in keratinocyte differentiation in AD skin result in hyperplasia of the basal layer, reduction of spinous layer and inhibition of markers of terminal differentiation (FLG, IVL, LOR) in AD **(B)** as compared to NA skin (**A**) (reproduction with permission from reference 4 and reference 134). Schematic of epidermal differentiation pattern in normal **(C)** and AD skin **(D)** is shown. The keratinocyte differentiation process is an integrated multi-step program of sequential changes in gene expression and cell structure, as the cells migrate from the proliferative basal layer, through spinous and granular layers, into the cornified layer, which functions as a skin barrier. Cells proliferate in the basal layer of epidermis. In the spinous (suprabasal) layer, cells irreversibly exit the cell cycle and switch from KRT5/KRT14 to KRT1/KRT10 production. Wnt/beta-catenin pathway is active in the proliferating epidermis, while keratinocyte differentiation in the spinous layer is under control of the Notch pathway. Deficient Notch activity alters epidermal differentiation in AD skin, expanding the proliferative compartment and influencing subsequent changes in epidermal

differentiation program. Changes in extracellular Ca2+ and lipid metabolism trigger the protein kinase C (PKC) pathway activation and regulates transcription of late differentiation markers in granular layer, FLG, LOR, IVL, HBD and TGM1. Inhibition of terminal differention marker expression is observed in AD skin.
Permissions obtained from reference 28 and reference 134 to publish edited portions of images.

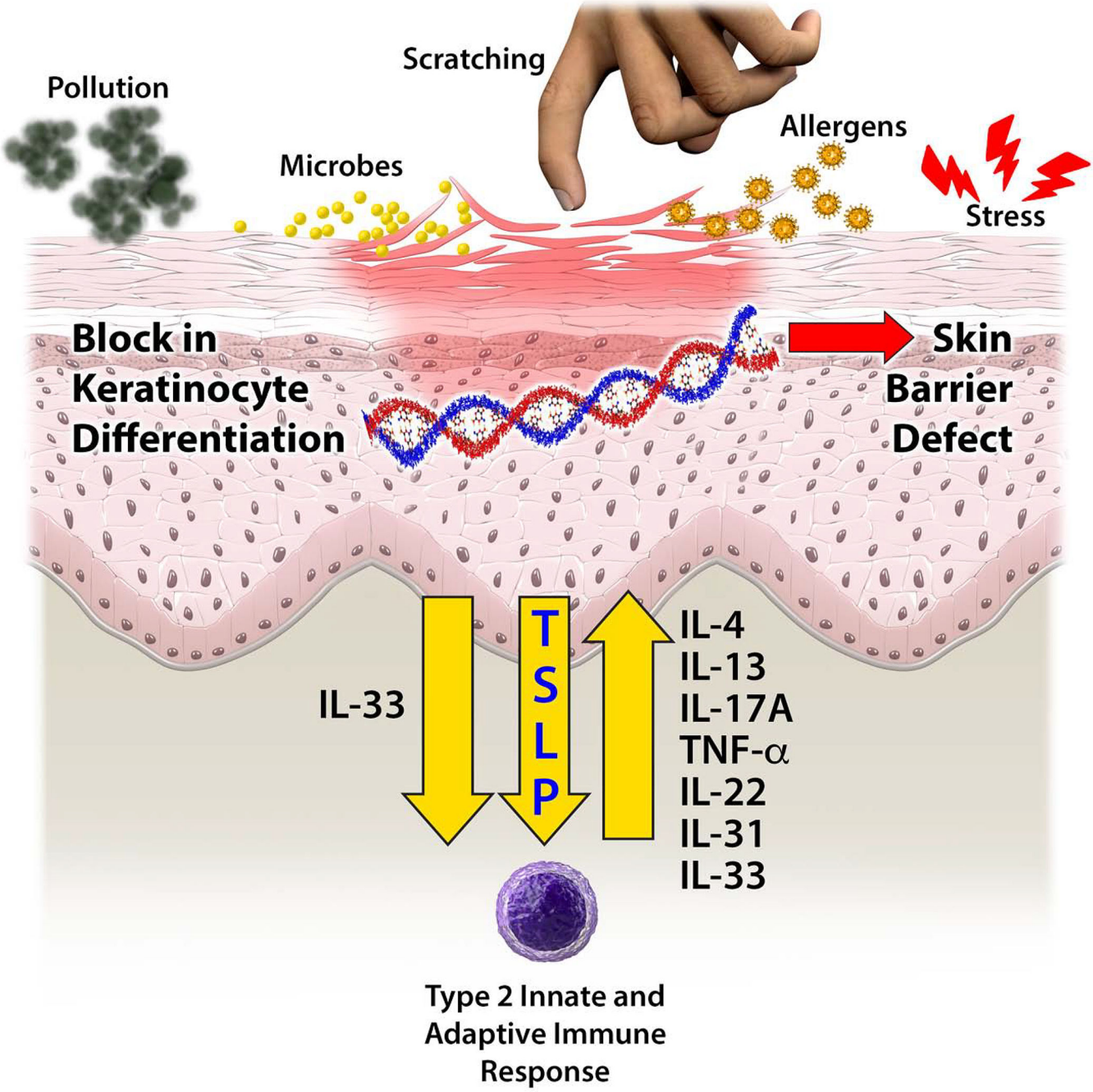


Figure 3. Causes of Cutaneous Barrier Dysfunction in AD and Allergic Diseases.

This includes genetic mutations, environmental influences (including microbes, scratching allergens) and the immune response (including IL4, IL13, IL17A, TNF-α, IL22, TSLP, IL31, IL33).

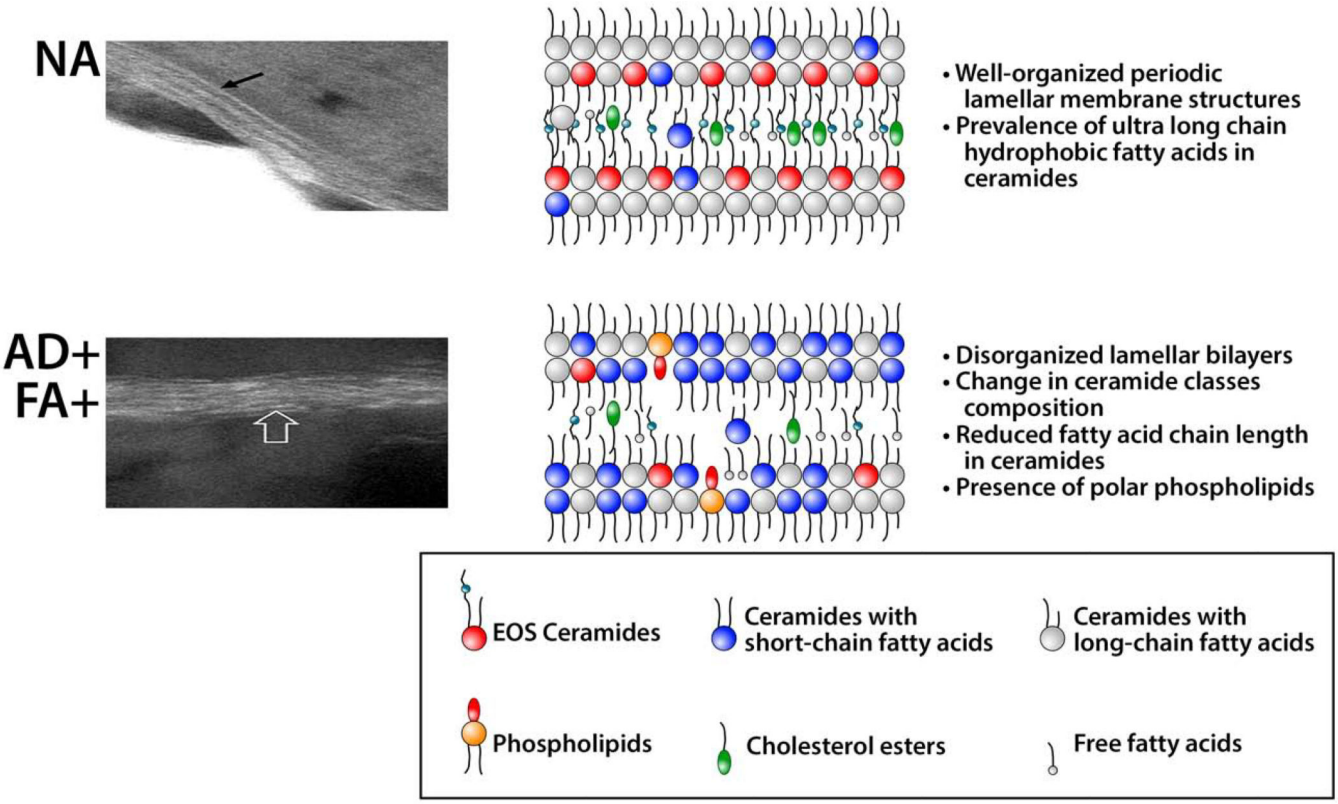


Figure 4. Lipid Barrier Abnormalities in AD Skin.

The intercellular lipids (the “mortar”) are an integral component of the stratum corneum skin barrier. They consist of a heterogeneous mixture of ceramides, free fatty acids, and cholesterol. These lipids are produced in the stratum granulosum and stored in lamellar bodies, and then secreted into extracellular space in the transition to the stratum corneum. In healthy skin nonatopic (NA), lipid lamellae are well organized; EOS Ceramides and ceramides with ultra long-chain fatty acids form a crystalline structure. In AD skin, the increase in ceramides with short-chain fatty acids as well as the presence of polar lipids disrupt lamella structure. Gaps in lipid lamellae structures of AD skin may support penetration of allergens and water loss through skin barrier. Electron microscopy photographs of NA and AD skin are reproduced from previously published work (120). Permission obtained from reference 120 to publish edited portions of images.

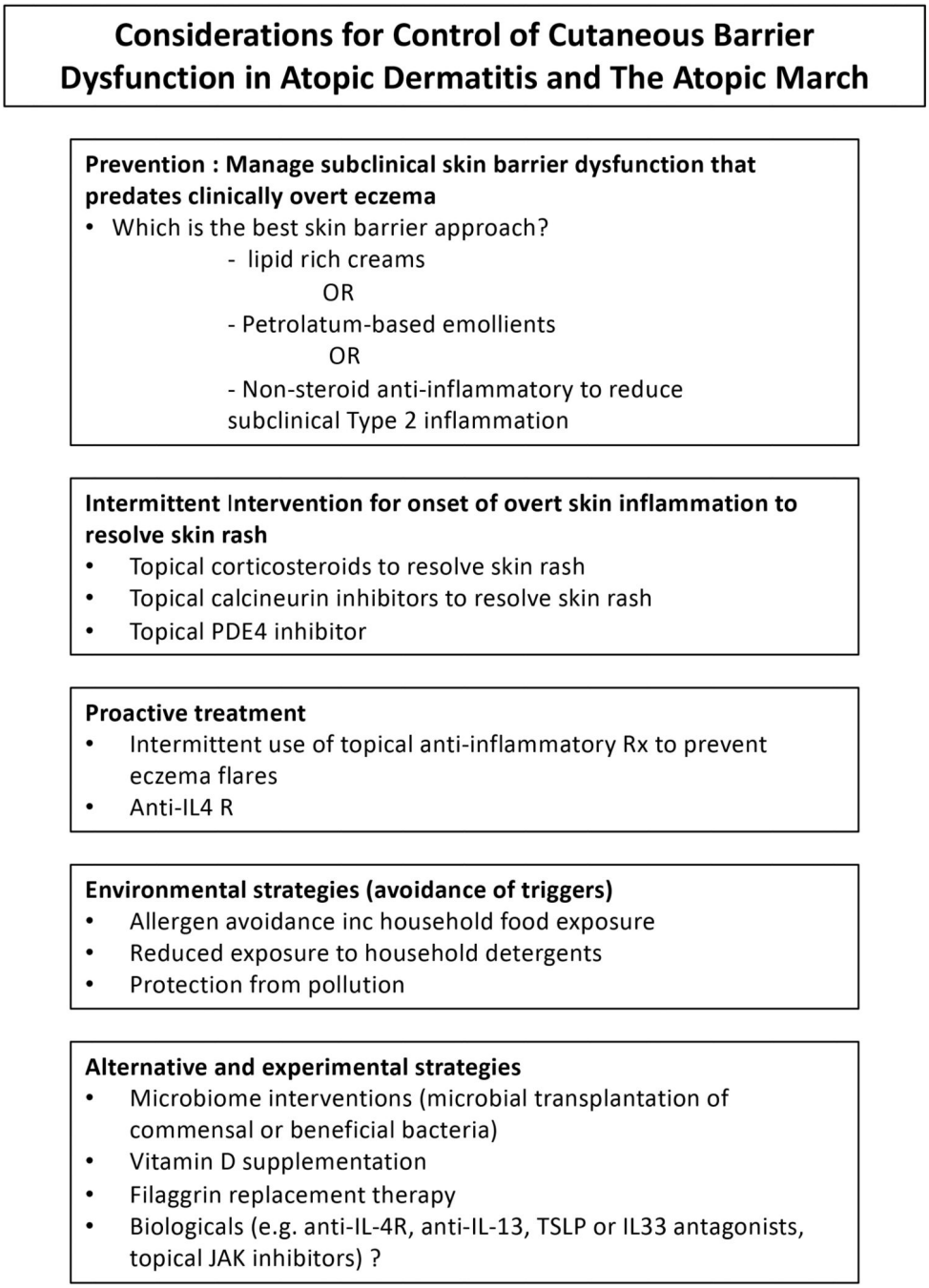


Figure 5.
Management Strategies for the Prevention and Treatment of AD and The Atopic March