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Atopic dermatitis: a disease of altered skin barrier and immune dysregulation

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Summary: Atopic dermatitis (AD) is an important chronic or relapsing inflammatory skin disease that often precedes asthma and allergic disorders. New insights into the genetics and pathophysiology of AD point to an important role of structural abnormalities in the epidermis as well as immune dysregulation not only for this skin disease but also for the development of asthma and allergies. Patients with AD have a unique predisposition to colonization or infection by microbial organisms, most notably *Staphylococcus aureus* and herpes simplex virus. Measures directed at healing and protecting the skin barrier and addressing the immune dysregulation are essential in the treatment of patients with AD, and early intervention may improve outcomes for both the skin disease as well as other target organs.

Keywords: atopic dermatitis, antimicrobial peptides, eczema herpeticum, epidermal barrier, filaggrin, *Staphylococcus aureus*

Introduction

Atopic dermatitis (AD) is an important common chronic or relapsing inflammatory disease of the skin that often precedes asthma and allergic disorders (1, 2). Lifetime prevalence in school-aged children in the USA has been reported to be up to 17% (3). The wide range of prevalence (8.7–18%) reported in the most recent US data derived from the 2003 National Survey of Children's Health (4) suggests that environmental or other unidentified factors may influence disease expression. High prevalence rates have also been observed in a number of other countries, with data from over a million children in 97 countries showing that AD is a major problem in developing as well as developed countries (5, 6). Atopy, the genetic predisposition to make IgE antibody responses to common environmental or food protein antigens, has been shown to be strongly associated with AD with an incidence of approximately 80% in infants with AD (7). Of note, an 'atopic march' occurs early, as >50% of children with AD will develop asthma and/or allergies, typically by their third birthday (8). Patients with severe or persistent disease and their families experience significant impairment in their quality of life (9),

and in addition, AD places a heavy economic burden on society as a whole (10, 11). This review highlights recent insights into the genetics and pathophysiology of AD focusing on the relationship between skin barrier abnormalities and immune dysregulation.

Genetics of AD: an evolving story

AD is a highly heritable disease with phenotype-specific genes likely playing an important role along with 'generic' atopy genes. In a large birth cohort study, the risk of a child having AD if one or both parents had AD was found to be higher [odds ratio (OR), 3.4; 95% CI, 2.6–4.4] compared with the risk if one or both parents had asthma (OR, 1.5; 95% CI, 1.0–2.2) or allergic rhinitis (OR, 1.4; 95% CI, 1.1–1.8) (12). However, the relatively low concordance observed in some studies, even for identical twins, points to the role of environmental factors influencing disease risk and manifestation (13). The approach to identifying genes associated with AD has included genome-wide linkage and candidate gene association studies (reviewed in 14). Five genome-wide linkage studies performed on AD plus a genome-wide linkage screen originally designed for asthma with analyses repeated for the AD outcome were all performed on families of European ancestry, except for one that was performed on Japanese families. Linkage to AD was found on chromosomes 1, 3, 4, 5, 11, 13, 15, 17, 18, 19, and 20 with only the 3p24 locus showing significant replication. Although the genome-wide linkage approach has identified a number of candidate genes associated with AD, none has conclusively pinpointed a specific locus or gene. It is also worth noting that no candidate gene was identified with positional cloning in the AD genome-wide linkage studies (14). In contrast, a candidate gene approach focusing on a specific gene or set of genes believed to be causally involved in the underlying pathology of a certain disease is not limited to families and can be applied to case-control study designs, which possess certain advantages over family-based studies and are generally considered to be more powerful in detecting true associations once the gene has been identified (15). Although >100 studies have reported an association of AD and a candidate gene, most of these were insufficiently powered, and heterogeneity of the AD phenotype in the studies makes replication of the observed associations difficult. Nevertheless, with this approach, of 81 genes examined, 46 had at least one positive association study reported. Of these 46 genes, 13 (FLG, IL4, IL4RA, SPINK5, CMA1, IL13, RANTES, CD14, DEFB1, GSTP1, IL18, NOD1, TIM1) were positively associated in at least one other independent study with the gene encoding

filament-aggregating protein, FLG, associated with AD the highest number of studies (14). The genes identified encode for proteins predominantly involved in skin barrier function as well as innate and adaptive immune responses.

In a recent study, looking at the association between prenatal farm-related exposures and AD in a birth cohort, the authors also analyzed the association between the expression of innate immune genes at birth and AD (16). In this prospective study, gene expression of TLRs and CD14 was assessed in cord blood leukocytes by quantitative polymerase chain reaction. Maternal contact with farm animals and cats during pregnancy had a significantly protective effect on AD in the first 2 years of life. The risk of AD was reduced by more than half among children with mothers having contact with three or more farm animal species during pregnancy compared to children with mothers without such contacts. Elevated expression of TLR5 and TLR9 in cord blood was associated with a decreased diagnosis of AD. In addition, a significant interaction between polymorphism in TLR2 and prenatal cat exposure was observed in AD.

In a novel approach, Barnes (14) evaluated 81 genes reported to have association with AD using the Ingenuity Pathway Analysis (www.ingenuity.com). More than half of the genes studied were clustered in two major networks associated with immune dysregulation: the pathway associated with antigen presentation, cell-mediated and humoral immune responses, and the pathway associated with cell signaling and interaction, cellular movement, and hematologic system development and function (Table 1). CD14 (monocyte differentiation antigen), GATA3 (GATA-binding protein 3), IL4, IL18, NOD1 (nucleotide-binding oligomerization domain 1), and TLR2 (Toll-like receptor 2) genes, previously shown to be significantly associated with AD, were clustered in the antigen presentation and immune response pathway, whereas BCL2A1 (BCL2-related protein A1), BDNF (brain-derived neurophilic factor), RANTES (regulated upon activation, normally T-expressed, and presumably secreted), CSF2 (colony-stimulating factor 2), GSTP1 (glutathione-S-transferase 1), IL5, IL12B, IL12RB1, and SOCS3 (suppressor of cytokine signaling 3) genes were clustered in the cell-signaling or movement pathway. This approach may prove especially useful in selecting optimal candidates for further genetic association studies.

Patients with AD have an increased susceptibility to infections and cutaneous colonization (2) and several immune function genes have been shown to be associated with this predisposition. Gene association studies on AD implicate TLR2, NOD1, NOD2, CD14 and DEFB1 (β -defensin 1). It is important to recognize that these and other AD-associated genes discussed

Table 1. Genes involved in immune dysregulation associated with atopic dermatitis (from ref. 14)

Antigen presentation, cell-mediated and humoral immune response pathway	Cell signaling and interaction, cellular movement and hematologic system development, and function pathway
<i>CD14</i> (monocyte differentiation antigen)	<i>BCL2A1</i> (BCL2-related protein A1)
<i>GATA3</i> (GATA-binding protein 3)	<i>BDNF</i> (brain-derived neurotrophic factor)
<i>IL4</i>	<i>RANTES</i> (Regulated upon Activation, Normally T-Expressed, and presumably Secreted)
<i>IL18</i>	<i>CSF2</i> (colony-stimulating factor 2)
<i>NOD1</i> (nucleotide-binding oligomerization domain 1)	<i>GSTP1</i> (glutathione-S-transferase 1)
<i>TLR2</i> (Toll-like receptor 2)	<i>IL5</i>
	<i>IL12B</i>
	<i>IL12RB1</i>
	<i>SOCS3</i> (suppressor of cytokine signaling 3)

above are not necessarily unique to AD. A number of AD candidate genes are associated with diseases of immune dysregulation including asthma and allergies, consistent with the hypothesis that certain disease genes are not disease specific, but contribute to related clinical phenotypes (17).

Linkage studies performed in AD have implicated loci containing clusters of genes associated with skin barrier dysfunction including those found in the epidermal differentiation complex (EDC) on chromosome 1q21 encoding epidermal cornification and S100 proteins (18). Of these, *FLG* has been the most widely studied and as discussed previously, to date *FLG* is the gene with the highest association with AD. In a landmark paper published in 2006, Palmer et al. (19) showed that in a European Caucasian population, homozygous nonsense mutations in *FLG* (R501X and 2282del4) that stop protein translation were strongly associated with AD. A growing number of additional mutations have been reported with varying frequency in various ethnic groups including Asian and African-American patients (20–23). A meta-analysis of *FLG* R501X and 2282del4 mutations in 6448 cases, 26 787 control subjects, and 1993 families all selected for AD confirmed that the two null mutations represent the strongest and most compelling genetic risk factors for AD (24). Beside mutations in *FLG*, genomic profiling of mRNA in AD has identified defects in expression of multiple genes encoding the cornified envelope, including *loricrin* (25) (Fig. 1).

The study by Gao et al. (22) showed that the frequency of the *FLG* R501X mutation was three times higher and the rela-

tive risk for disease nearly double for AD patients with eczema herpeticum (EH), a serious viral complication, compared to AD without EH (OR, 11.8 versus 6.2; $P = 0.0008$). This is the first study providing insight into the genetic associations of AD and complicating factors, suggesting that skin barrier dysfunction associated with *FLG* mutations contributes to the increased propensity for disseminated viral skin infections seen in a subset of AD patients. More recently, a multicenter case–control study demonstrated an association between markers in thymic stromal lymphopoietin (TSLP) and its receptors, *IL7R* and *TSLPR*, and risk of AD and EH (26). The findings point to TSLP as an important candidate for AD, AD severity, and ADEH and are the first to implicate TSLP as a potential casual gene for ADEH. Patients with AD are also at high risk to develop serious, potentially life-threatening reactions to vaccinia, the attenuated virus used for smallpox vaccination (27, 28). In a gene-profiling study of skin biopsy specimens to vaccinia virus (VV), Grigoryev et al. (29) found differences in the transcriptional response to VV in AD patients compared with patients with psoriasis or healthy subjects. The unique molecular signature in AD skin included genes involved in the innate immune response and wound healing.

More recently, genome-wide association studies (GWASs) have been introduced as an unbiased approach to search for genes controlling the risk for complex diseases (30). Results from the first GWAS in AD identified two novel genetic variants in association with eczema susceptibility: a single-nucleotide polymorphism on chromosome 11q13.5 (rs7927894) and a single-nucleotide polymorphism (rs877776) within the gene encoding hornerin on chromosome 1q21 (31). Subsequently, a case–control study investigated the association of rs7927894, rs877776, and the four most prevalent *FLG* null mutations with moderate–severe eczema in 511 Irish pediatric cases and 1000 Irish controls (32). Comprehensive testing for interaction between each of the loci was also performed. The association between rs7927894 and AD was replicated in this population ($P = 0.0025$; OR, 1.27; 95% CI, 1.09–1.49), but no significant association between rs877776 AD was found. The four most common *FLG* null variants were strongly associated with AD [$P = 1.26 \times 10^{-50}$; combined OR, 5.81; 95% CI, 4.51–7.49]. The rs7927894 association was found to be independent of the *FLG* risk alleles and may be multiplicative in its effect. Thus, the single-nucleotide polymorphism rs7927894 appears to identify a genuine eczema susceptibility locus that will require further elucidation through fine mapping and functional analysis.

Complex interactions have been described between immune abnormalities characterizing AD such as elevated Th2

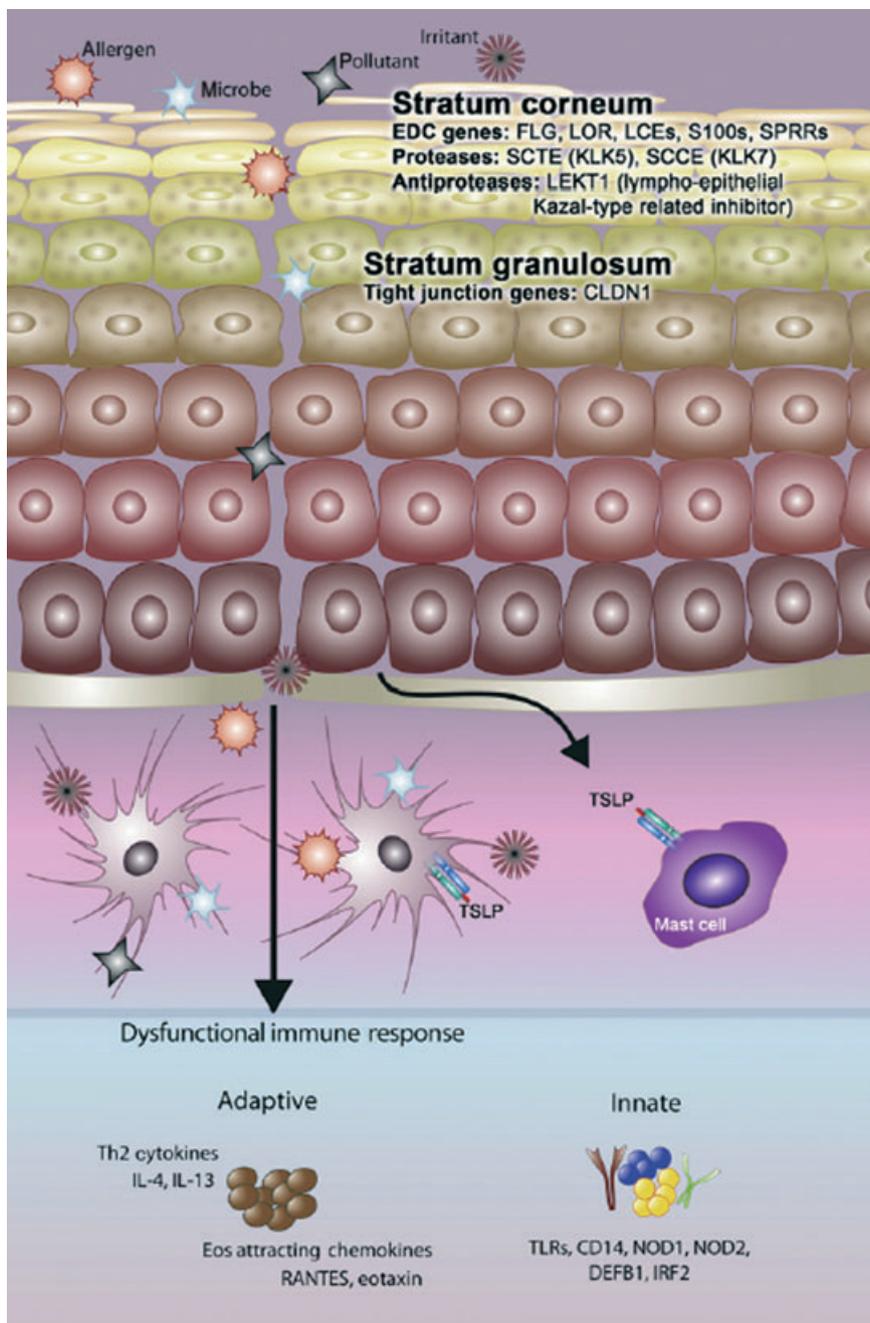


Fig. 1. Complex pathophysiology of atopic dermatitis including barrier and immune abnormalities. Reproduced with permission from Barnes (14).

cytokine levels (1) and expression of genes involved in epidermal barrier function (33, 34). In a murine model, IL-4 was shown to decrease the expression of multiple genes associated with innate defense, including genes in the EDC that regulate epidermal barrier function. Mice that express a constitutively active Stat6 (Stat6VT) are prone to the development of allergic skin inflammation and have decreased the expression of EDC genes. IL-4 deficiency was shown to protect

Stat6VT transgenic mice from developing allergic skin inflammation and to decrease the time to recover barrier function with a concomitant increase in EDC gene expression (35). Insights from genetic studies suggest that immunologic and skin barrier-related genetic variations may work synergistically to increase susceptibility to AD. In addition to susceptibility, *FLG* null alleles have also been shown to predispose to early-onset AD that persists into adulthood, thus defining a

possible unique phenotype of AD (36). Furthermore, patients 50 years of age or older with AD are at increased risk for having *FLG* mutations (37).

***FLG* mutations and risk of asthma and allergic disorders**

Studies of *FLG* mutations have pointed to an important association of skin barrier abnormalities in the development of asthma and allergies. In the initial study that showed an association between AD and *FLG* mutations, Palmer *et al.* (19) also showed that these mutations were strongly associated with asthma, but only in those individuals who had eczema. Subsequent studies have confirmed this important association (24, 38–40). Other studies have shown the association of *FLG* mutations with allergic sensitization and allergic disorders (38, 39, 41) and support the concept of an atopic march with AD followed closely by the development of allergies and asthma (42). In a prospective clinical study from birth to school-age, a high-risk cohort of European children was assessed for the development of AD, asthma, and allergies (43). Two common *FLG* variants were evaluated in Caucasian children and these were found to significantly increase risk of eczema, as well as developing recurrent wheeze, asthma, and asthma exacerbations [hazard ratio 1.82 (1.06–3.12), $P = 0.03$] expressed within the first 1.5 years of life. The children with *FLG* mutations had a marked and persistent increase in acute severe asthma exacerbations from 1 year of age [incidence ratio 2.40 (1.19–4.81), $P = 0.01$] and increased risk of asthma by age 5 [OR, 2.62 (1.12–6.11), $P = 0.03$]. *FLG* variants also increased risk of specific sensitization by age 4 [OR, 3.52 (1.72–7.25), $P = 0.0007$] but not age 1.5. Although these associations have been described in predominantly European Caucasian populations, Imoto *et al.* (44) recently reported allergic sensitization associated with a *FLG* gene mutation in an Asian population. Of interest, in infants with eczema and sensitization to food allergens, *FLG* mutations were shown to predict childhood asthma with a positive predictive value of 100% (95% CI, 65.5–100) (45). These findings point to a strong synergistic interaction between *FLG*-null alleles and early food sensitization in the disease transition from eczema to asthma (relative excess risk due to interaction, 2.64; 95% CI, 1.70–3.98; $P = 0.00040$) and suggest that *FLG* mutations and food sensitization represent distinct but closely interacting mechanisms in the development of asthma. Genotyping infants with eczema and food sensitization for *FLG* mutations could allow for the prediction of asthma before the onset of symptoms and facilitate the development of early subgroup-specific interventions to prevent progression in the atopic march.

As filaggrin was not detected in bronchial biopsies from asthmatic and normal volunteers (46), this finding suggests that this skin barrier protein is not directly involved in lower airway permeability to environmental allergens. In addition, filaggrin was not expressed in nasal or esophageal epithelium (47) and is therefore unlikely to play a role in barrier function at these mucosal surfaces. The hypothesis that allergic sensitization occurs through a damaged skin barrier and leads to local and systemic immune responses (48) is supported by studies with an *FLG*-deficient mouse (49), as discussed below. Of interest, development of peanut allergy has been linked to transcutaneous exposure to peanut allergens in topical preparations (50) and in the environment (51), and early onset AD is a risk factor for peanut allergy (50). Whether *FLG* or other barrier-specific abnormalities will be linked to increased risk for peanut or other food allergy remains to be seen.

As noted by Barnes (14), very few of the genetic association studies in AD have looked at associations between genetic polymorphisms and environmental factors. Data from two birth cohorts demonstrated a significant interaction between *FLG* loss-of-function mutations and cat ownership at birth on the development of early-life eczema (52). These findings suggest that cat but not dog ownership substantially increases the risk of eczema in the first year of life in children with *FLG* mutations but not in those without such mutations and points to the need for early identification of at-risk individuals with appropriate environmental control measures. These findings are in contrast to the study of animal exposures and innate immune genes in AD discussed earlier (16).

Moving beyond genomics

After genomics, proteomics is considered the next step in the study of biological systems (53). Using a proteomic approach, Howell *et al.* (54) found the S100 calcium-binding protein A11 (S100/A11) to be significantly downregulated in the presence of IL-4 and IL-13 in AD. S100/A11, an important component of the cornified envelope encoded within the EDC on chromosome 1q21 (55), was also shown to be decreased in the skin of AD patients. In addition, the authors also showed an immunomodulatory effect of S100/A11 on *FLG* and HBD-3 gene expression pointing to immune dysregulation effecting both epidermal barrier integrity and innate immune response. Using a novel mass spectrometry-based proteomics approach on skin tape strippings, Broccardo *et al.* (56, 57) found that patients with AD have unique epidermal protein profiles. Proteins related to the skin barrier and generation of natural moisturizing factor (NMF) were expressed at

significantly lower levels in lesional versus non-lesional sites of patients with AD with and without history of EH whereas epidermal fatty acid-binding protein was expressed at significantly higher levels in patients with methicillin-resistant *Staphylococcus aureus* (MRSA). Lower expression of skin barrier proteins and enzymes involved in the generation of NMF could exacerbate barrier defects and perpetuate water loss from the epidermis, whereas greater expression of epidermal fatty acid-binding protein, especially in patients colonized with MRSA, could perpetuate the inflammatory response through eicosanoid signaling.

Consequences of epidermal barrier abnormalities in AD

An intact, healthy skin barrier is a critical first line of defense against various microbes, irritants, and allergens. As discussed above, the epidermis of AD patients is characterized by significant barrier disruption, and AD patients have an increased susceptibility to allergic sensitization as well as microbial colonization and infections (2, 58). In normal subjects, formation of the cornified cell envelope involves dephosphorylation and cleavage of the large profilaggrin protein by serine proteases ending in the release of functional filaggrin (59). Filaggrin aggregates the keratin cytoskeleton to facilitate the collapse and flattening of keratinocytes in the outermost skin layer. Additionally, other proteins encoded by genes in the EDC discussed above including loricrin and involucrin are essential components of the epidermal barrier (60). As the water content of the stratum corneum drops, filaggrin is proteolyzed into pyrrolidine carboxylic acid and trans-urocanic acid that contribute to the composition of NMF (61). These breakdown products in turn act as osmolytes, accounting in large part for corneocyte hydration. The cellular basis for a permeability barrier abnormality remains to be fully elucidated (62). However, the most immediate result of FLG deficiency in AD is decreased stratum corneum hydration, resulting in a steeper water gradient across the stratum corneum and driving increased transepidermal water loss. In addition, filaggrin breakdown products play an important role in acidifying the stratum corneum and decreased generation of filaggrin metabolites could result in an increase in the stratum corneum pH of the stratum corneum with activation of a number of serine proteases (58). A pH-induced increase in serine protease activity could lead to both barrier breakdown and precipitate Th2 inflammation in AD (62). A recent *in vitro* study demonstrated that *S. aureus* growth rate and cell density were affected by the acidic filaggrin breakdown products urocanic acid and pyrrolidone carboxylic acid (63). Lower pH

was associated with reduced expression of secreted and cell wall-associated proteins, including proteins involved in colonization such as clumping factor B and fibronectin-binding protein A, as well as in immune evasion such as protein A. Importantly, exogenous proteases from *S. aureus* and dust mites could further perpetuate barrier and immune abnormalities (58) (Fig. 2).

Whether skin barrier dysfunction precedes skin inflammation and epidermal barrier defects initiate the development of AD remains an unresolved issue. In a recent study, infants were evaluated for evidence of skin inflammation and barrier dysfunction at the age of 3 months (64). Those with FLG mutations were more likely to have AD than infants without defined FLG mutations. Importantly, infants with FLG mutations had abnormal epidermal barrier function compared to infants without defined FLG mutations, even in the absence of clinically evident eczema. Although these results suggest that FLG mutations are associated with abnormal skin barrier function prior to the development of clinically overt inflammation, definitive proof of whether skin barrier dysfunction precedes skin inflammation and whether epidermal barrier defects are the key initiating factors in the development of AD awaits more sensitive analyses performed at multiple time points.

Skin barrier function as assessed by TEWL is intrinsically compromised in children with AD but not in children with other allergic conditions and also differs by race (65). The magnitude of skin barrier dysfunction correlates with AD disease severity. Skin barrier abnormalities may occur in AD patients irrespective of FLG genotype, suggesting that other factors may be important in modulating skin barrier integrity (66). Enhanced TEWL and diffusivity in AD may reflect defects in the intercellular lipid bilayers of the stratum corneum. However, disturbance of lipid organization in the stratum corneum could be impacted differently in AD patients with or without FLG mutations. Investigation of intercellular lipids of the stratum corneum in AD patients characterized for FLG genotype could provide further insights.

Lessons from mouse models

A number of mouse models of AD have been developed (reviewed in 67). These include eczema induced by epicutaneous application of sensitizers, transgenic mice overexpressing or lacking selective molecules, as well as mice that spontaneously develop AD-like skin lesions. More recently, Fallon et al. described a 1-bp deletion mutation, 5303delA, analogous to common human FLG mutations within the murine Flg gene in the spontaneous mouse mutant flaky tail (ft)

(49). Topical application of allergen to mice homozygous for this mutation resulted in cutaneous inflammatory infiltrates and enhanced cutaneous allergen priming with the development of allergen-specific antibody responses. These results support the flaky tail mouse as an important model of filaggrin deficiency. In addition, they support the hypothesis that antigen transfer through a defective epidermal barrier is a key mechanism underlying elevated IgE sensitization and initiation of cutaneous inflammation in humans with filaggrin-related atopic disease. Filaggrin-deficient *ft/ft* mice were also shown to exhibit TH17-dominated skin inflammation and eczematous changes with age as well as epicutaneous sensitization with protein antigen (68). The *ft/ft* mouse should provide new insights into the relationship of human FLG mutations and immune abnormalities in AD.

Digging deeper: tight junction abnormalities in AD

In addition to the stratum corneum, tight junctions found on opposing membranes of stratum granulosum keratinocytes directly below the stratum corneum form a second barrier (Fig. 1). Tight junctions are made up of a complex of adhesive and scaffolding proteins that control the passage of water, ions, and solutes through the paracellular pathway (69). The non-lesional epithelium of AD subjects has been shown to have bioelectric abnormalities indicative of a tight junction defect that could be the consequence of reduced levels of claudin-1 (CLDN1), a key tight junction adhesive protein (70). This is consistent with earlier work in KO mice that established the importance of epidermal tight junctions and claudin-1. CLDN1 knockout mice died within 24 h of birth with wrinkled skin, severe dehydration, and increased epidermal permeability as measured by dye studies and transepidermal water loss (71). In human AD studies, CLDN1 expression was shown to be inversely correlated with total circulating eosinophil counts and serum IgE, markers of Th2 polarity (70). This suggests that TJ defects may promote Th2 polarity, a characteristic of EH subjects (72). More recent mechanistic and genetic studies confirmed the critical role of tight junctions in maintaining barrier integrity and implicated reductions in claudin-1 levels in the spread of epidermal viral infections including EH (73).

Local and systemic immune dysregulation characterizes AD

A number of well-characterized systemic and cutaneous immune abnormalities have previously been described in AD, including increased serum IgE and sensitization to allergens,

elevated Th2-type cytokine expression in acute lesions, increased numbers of T cells expressing cutaneous lymphocyte-associated antigen (CLA) (the homing receptor for the skin), increased expression of FcεRI on both Langerhans cells and inflammatory dendritic epidermal cells, as well as decreased expression of anti-microbial peptides (reviewed in 2). Whether skin barrier abnormalities precede immune dysregulation ('outside-in' hypothesis) (74) or immune dysregulation precedes barrier changes ('inside-out' hypothesis) continues to be an unresolved argument with data supporting both hypotheses. It is important to recognize that not all patients with AD have FLG mutations, and conversely, individuals with FLG -null alleles may not develop AD (59). In addition, although the natural history of AD shows that adults with AD are more likely to have FLG mutations (37), other studies show that some of these patients do outgrow their disease or go into an extended remission, although they tend to have a more prolonged course than those patients without FLG mutations (38).

A critical link between the barrier defect in AD patients with FLG mutations and Th2 polarization could be explained in part by enhanced allergen penetration through the damaged epidermis accompanied by increased production of TSLP by keratinocytes, leading to a Th2-type milieu. TSLP has been called a 'master switch for allergic inflammation' (75) and shown to exert effects on a number of key cells involved in cutaneous inflammation, including mast cells, basophils, and eosinophils (reviewed in 76). AD patients with more polarized Th2-type disease with associated asthma and allergies as well as increased biomarkers, including TSLP, cutaneous T cell-attracting chemokine, and serum IgE, were more likely to have severe skin disease complicated by bacterial and viral skin infections (77). TSLP was recently shown to be induced in the epidermis after mechanical stimulation (78) and plays a role in activation of Th2 cytokine-secreting invariant natural killer cells, which are increased in AD skin (79). Polymorphisms of the TSLP gene have been shown to contribute to Th2-polarized immunity through higher TSLP production (80). As discussed previously, genetic variants in TSLP were recently shown to be associated with AD and EH (26). Still, the relationship of skin barrier and immune abnormalities to the increased susceptibility to microbial colonization and infections remains to be fully elucidated (81). Observations that topical calcineurin inhibitors can partially correct the barrier defect in AD and that gentamicin can restore the production of functional filaggrin chains provides further evidence of the complex relationship of the epidermal barrier and the immune system (82).

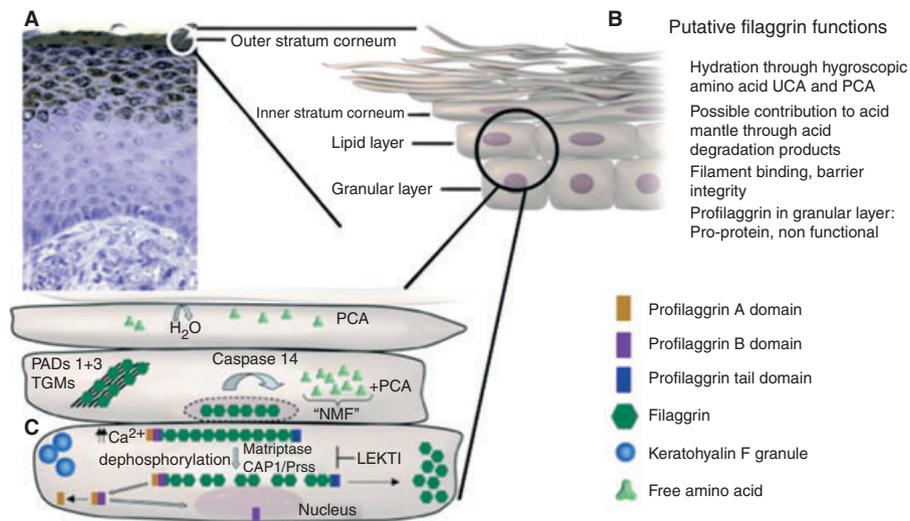


Fig. 2. Filaggrin expression and proposed functions. Reproduced with permission from O'Regan et al. (59).

New subsets of T cells in AD

Naturally occurring CD4⁺CD25⁺ FoxP3⁺ T-regulatory (Treg) cells with normal immunosuppressive activity appear to be expanded in the peripheral blood of patients with AD (83). However, after stimulation by the superantigen (SAg) staphylococcal enterotoxin B (SEB), Treg cells appear to lose their immunosuppressive activity. This observation suggests a novel mechanism by which SAGs could augment T-cell activation in patients with AD (84). Although increased numbers of Treg cells in the peripheral blood of patients with AD have been confirmed (85) and related to inflammatory signals with Treg cell-suppressive activity subverted by proinflammatory mediators, CD4⁺CD25⁺FoxP3⁺ Treg cells were not found in lesional AD skin or in atopy patch test sites of AD patients (86). These studies contribute to previous observations of a number of discordant systemic versus skin-specific immune abnormalities (1).

The role of IL-17-secreting Th17 cells in AD has been investigated in several studies. IL-17 was shown to be preferentially elevated in acute versus chronic AD lesions (87). In addition, IL-17 expression was induced in the skin as well as in the airways in a murine epicutaneous antigen challenge model analogous to human AD (88). As discussed above, this group also showed that filaggrin-deficient mice exhibited Th17-dominated skin inflammation and eczematous changes with increased expression of IL-17 in the epidermis and increased antigen-specific IgE in their serum (68). In an atopy patch test model, IL-17 secretion was shown to be enhanced by SEB (89). Induced IL-17 upregulated the anti-microbial peptide HBD-2 in human keratinocytes *in vivo*, although co-expressed

IL-4/IL-13 partially inhibited this effect. Thus, although IL-17-secreting T cells have been shown to infiltrate acute AD lesions and IL-17 secretion can be triggered by SAGs, ineffective IL-17-dependent upregulation of HBD-2 in patients with AD may result from partial inhibition by the Th2 cytokine milieu. A study in IL-4 and IL-13 knockout mice supports a role for IL-4 as a Th2 cytokine that downregulates the IL-17 response in epicutaneously sensitized mice (90). This abnormality could in part be responsible for the persistent colonization by *S. aureus* characteristically observed in AD patients. In addition, compared with psoriasis, a chronic inflammatory skin disease with abnormal barrier but without the predisposition to skin infections or colonization, Th17 cells appear to have a diminished role in AD skin and the associated reduced expression of innate immune defense genes may contribute to increased skin infections in AD (91). Both IL-4 and IL-13 were recently found to significantly antagonize IL-17 induction of anti-microbial genes such as *DEFB4* (92). These findings have possible therapeutic implications, as measures directed at increasing signaling through intact IL-17 receptors, despite low ligand expression, could potentially reverse the relative AMP deficiency seen in AD (93). Neutralizing Th2 cytokines as previously demonstrated in skin explants from AD patients (94), thus allowing existing IL-17 to function, could potentially restore AMP expression and decrease microbial colonization, although such an approach could tip the balance toward AMP-driven inflammation. In addition, data from the same group showed that T cells could independently express IL-22 even with low expression of IL-17 (95). This observation suggests that T cells could have functional specialization with 'T17' T cells inducing AMP and 'T22' T cells

driving epidermal hyperplasia. Further characterization of T22 T cells is warranted and may have clinical as well as therapeutic implications.

Immunologic basis for pruritus in AD

Pruritus is the major symptom of AD that impacts most significantly on the quality of life of patients. Anti-histamine therapy is frequently ineffective, suggesting that mediators other than histamine such as cytokines and neuropeptides may be involved (96). IL-31 is increased in AD skin lesions (97) and has been implicated in the development of chronic dermatitis in transgenic mice that overexpress IL-31 (98). Sources of IL-31 include skin-infiltrating CLA⁺ T cells and peripheral blood CD45RO CLA⁺ T cells (99). Irrespective of the atopic phenotype, serum IL-31 levels have been shown to correlate with disease activity in AD (100). *S. aureus*-derived SAgS have been shown to rapidly induce IL-31 mRNA expression in the skin of atopic individuals *in vivo* and in PBMCs *in vitro*, suggesting that chronic colonization and superinfection by this microbial organism can contribute to pruritus and inflammatory changes in AD (97).

Role of microbial organisms in AD

Patients with AD have a unique propensity to be colonized or infected by a number of microbial organisms (2). Dysregulation of the adaptive immune response with elevated total and specific IgE levels has been associated with disease severity and infectious complications (101, 102). Innate immune system abnormalities, including reduction in anti-microbial peptides, diminished recruitment of cells such as neutrophils to the skin, and Toll-like receptor defects (103) as well as epidermal barrier abnormalities discussed previously (58) play an important role in microbial colonization or infection in AD. In addition, the relationship between innate and adaptive immune responses with infectious complications of AD continues to be elucidated. Mobilization of HBD-3 and killing of *S. aureus* by keratinocytes from AD patients were both shown to be significantly inhibited by IL-4 and IL-13, whereas neutralization of these cytokines significantly improved these activities (104). Serum IgE levels in AD patients with HSV infections were shown to correlate inversely with cathelicidin LL-37 expression (72). How aberrations in adaptive and innate immune responses and barrier abnormalities interact in AD remains to be fully investigated.

S. aureus can be cultured from 90% of skin lesions and importantly can colonize normal appearing skin in AD (105). *S. aureus* can exacerbate or contribute to persistent skin inflam-

mation in AD by secreting toxins with superantigenic properties, resulting in marked activation of T cells and other immune cells (106). Application of SEB to the skin can induce eczematous changes accompanied by infiltration of T cells selectively expanded in response to the SAg (107). In addition, AD patients can make specific IgE antibodies directed against the toxins found on their skin with basophils from these patients releasing histamine on exposure to the relevant toxin (108, 109). This suggests that SAgS can induce mast cell degranulation after penetrating the epidermal barrier and contribute to pruritus and acute inflammatory events along with participating in chronic skin inflammation. *S. aureus* isolates from patients with steroid-resistant AD have been shown to produce increased numbers of SAgS compared with isolates from controls suggesting a possible selective advantage of SAgS for colonization of patients (110).

Other products of *S. aureus* can also contribute to clinical disease in AD. Patients with impetiginized AD were found to have elevated levels of lipoteichoic acid (LTA) that correlated with clinical scores and *S. aureus* colony forming unit (CFU) (111). The amounts of LTA in the skin lesions were sufficient to exert biological effects on various cell types *in vitro*, as well as epidermal cytokine gene expression when skin was exposed to LTA *ex vivo* providing a novel mechanism by which *S. aureus* can exacerbate AD.

MRSA has emerged as an important pathogen that has rapidly evolved from a cause of nosocomial to community-acquired infections (106). MRSA invariably produce SAgS, and AD patients may be particularly susceptible to colonization and infection by MRSA as well as to the inflammatory consequences of these SAgS, as they are frequently treated, often with extended courses with anti-staphylococcal antibiotics (112). Phenol-soluble modulins (PSMs) represent a novel class of secreted *S. aureus* peptides that contribute to the enhanced virulence of community acquired (CA)-MRSA (113). These peptides appear to target neutrophils, recruiting, activating and then lysing these cells. PSMs are under the regulatory control of the accessory gene regulator (*agr*) quorum-sensing system, which controls the expression of a number of staphylococcal virulence factors. Understanding the underlying mechanisms for infection and colonization by *S. aureus* of the skin of patients with AD including differences between methicillin-sensitive *S. aureus* versus MRSA is critical for developing more effective treatment strategies for this growing public health problem.

In addition to bacterial infections, a subset of AD patients is at risk for localized and disseminated cutaneous viral infections, most often caused by HSV (114). EH is a potentially

life-threatening infection due to disseminated HSV that occurs in 10–20% of patients with AD. Risk factors for EH include early onset of AD, severe and untreated AD, head and neck dermatitis, previous EH or HSV infections, elevated total serum IgE with higher level of allergic sensitizations (115). As discussed previously, AD patients with EH have more severe, Th2-polarized disease with greater allergen sensitization compared with AD patients without a history of EH (77). Patients with ADEH also have a defect in the inducibility of the AMPs, HBD-2, HBD-3, and cathelicidin (116). AD patients of both European and African ancestry with *FLG* R501X mutations have been found to have an even greater risk for EH (22), and genetic variants in *TSLP* have also been shown to be associated with ADEH (26), implicating both skin barrier and immune abnormalities as contributors to this serious complication. AD patients are also at risk for potentially life-threatening complications from VV used to vaccinate against smallpox (27, 28). Skin explants from AD subjects inoculated with VV showed increased viral replication compared to healthy controls (94) with low levels of LL-37 and increased expression of IL-4 and IL-13. Importantly, neutralizing antibodies against these Th2 cytokines inhibited VV growth and increased levels of LL-37. Further studies may provide insights into identifying which AD patients are at greatest risk for complications from immunization with VV.

Implications of skin barrier and immune abnormalities for novel therapeutic strategies in AD patients

Jensen et al. (117) looked at TEWL, as well as several other parameters of epidermal barrier including stratum corneum hydration and dye penetration, and showed improvement in all parameters when AD patients were treated with both topical steroid and topical calcineurin inhibitors. Electron microscopic evaluation of barrier structure showed prevalently ordered stratum corneum lipid layers and regular lamellar body extrusion in the calcineurin inhibitor treated skin but inconsistent extracellular lipid bilayers and only partially filled lamellar bodies in the steroid treated skin. Both treatments normalized epidermal differentiation and reduced epidermal hyperproliferation. Of note, although the expression of filaggrin was also reduced in patients with AD, it was completely restored on treatment with either anti-inflammatory therapy. This is consistent with studies showing that filaggrin and other barrier protein expression in AD were downregulated through Th2 cytokine activity (33, 34). Of interest, tumor necrosis factor- α (TNF- α) was recently shown to modulate filaggrin and loricrin protein expression via a c-Jun N-terminal kinase

dependent pathway (118). Psoriasis patients treated with a TNF- α antagonist had significant enhancement of epidermal barrier protein expression, suggesting that TNF- α acts to inhibit key skin barrier proteins and use of specific antagonists may improve barrier protein expression. Thus, specific immunomodulatory therapy could be of benefit in AD and other diseases with epidermal barrier abnormalities.

Vitamin D deficiency is being increasingly recognized as playing a role in allergic diseases (119). Vitamin D appears to also be involved in the regulation of AMPs in keratinocytes (120). The results of a trial with oral vitamin D in AD patients support this hypothesis (121). In addition, children with AD treated with oral vitamin D in a randomized, controlled trial showed clinical improvement versus placebo (122). Larger multicenter trials with oral vitamin D in AD are currently in progress. An analysis of the National Health and Nutrition Examination Survey 2001–2004 found that individuals with vitamin D deficiency had a statistically significant increased risk of MRSA carriage (123). Whether vitamin D supplementation can decrease MRSA colonization remains to be studied.

The emergence of CA-MRSA has created an urgency to develop novel strategies to combat this microbial organism. An important consideration in devising new therapeutic strategies against *S. aureus* is recognizing that protective immunity to staphylococcal infections does not appear to exist to any significant degree, due in part to the fact that our immune system is in constant contact with staphylococcal antigens and many strains are commensal organisms (106). In addition, *S. aureus* produces protein A to help it evade acquired host defense. Although several attempts to develop protective vaccines have met with failure in clinical trials (e.g. StaphVax), promising results based on using a combination of systematically selected antigens have been reported (124). These combinatory vaccines target microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), a family of bacterial proteins that bind to human extracellular matrix components. A vaccine based on a combination of antigens was shown to provide complete protection from lethal doses of *S. aureus* in a murine challenge model (125). Importantly, MSCRAMM vaccines have been shown to interfere with *S. aureus* colonization (126). Whether this will result in decreased infection rates remains to be determined. Another potential antigenic target for vaccine development against *S. aureus* is teichoic acid, which has been implicated in nasal colonization and biofilm formation (127). Patients with impetiginized AD have been found to have significant levels of LTA in infected lesions that were able to induce epidermal cytokine gene expression *ex vivo* (111). Trials with a

conjugated vaccine that includes both a-toxin and Panton-Valentine leukocidin are currently in progress. In addition, a phase I, multicenter, double-blind study of a novel vaccine containing the conserved *S. aureus* iron surface determinant B conducted to assess the immunogenicity and safety of an adjuvant formulation of V710 showed lasting immune responses in adult subjects with no immediate or serious adverse events reported (128).

As discussed previously, *S. aureus* secretes exotoxins with properties of SAGs and a novel approach involves engineering-binding affinity agents capable of neutralizing these SAGs. Soluble forms of the engineered V β proteins produced in *E. coli* were shown to be effective inhibitors of SEB-mediated T-cell activation and completely neutralized the lethal activity of SEB in animal models (129). Subsequently, the same group was able to express V β domains in tandem as a single-chain protein and neutralized SEB and TSST-1 with a single agent demonstrating the feasibility of engineering a broader spectrum antagonist capable of neutralizing multiple toxins (130). Such an approach may be especially relevant to AD, as *S. aureus* isolates from patients with steroid-resistant AD have been shown to produce increased numbers of SAGs compared with isolates from controls (110).

Although cathelicidins and HBD-3 exhibit potent antiviral activity against VV (131), their utility as clinically useful anti-VV agents is limited due to rapid degradation by endogenous tissue proteases. In contrast, ceragenins are synthetic anti-microbial compounds designed to mimic the structure and function of endogenous anti-microbial peptides (132) that can disrupt bacterial membranes without damaging

mammalian cell membranes (133). Due to their synthetic nature, ceragenins are not subject to human protease degradation allowing for a longer tissue half-life. One candidate compound (CSA-13) was found to exhibit potent antiviral activity against VV via direct antiviral effects and by stimulating the expression of endogenous anti-microbial peptides with known antiviral activity (134). Further topical application of CSA-13 in mice reduced VV-induced satellite lesion formation, suggesting that treatment with CSA-13 may be an intervention for patients with disseminated VV skin infection.

Conclusions

Patients with AD have genetically determined risk factors that affect their skin barrier function and immune responses and interact with environmental triggers. Clinically, this results in intensely pruritic, inflamed skin that allows the penetration of irritants and allergens and predisposes patients to colonization and infection by microbial organisms. New techniques such as GWAS and high-throughput expression profiling should greatly improve our ability to identify relevant gene polymorphisms, including rare variants in this complex disorder as well as other allergic diseases. Insights into the complex relationship between skin barrier and immune abnormalities should lead to more targeted therapy for AD and associated infectious complications. New methods to categorize unique phenotypes of AD may also lead to novel early interventions strategies that could also interrupt the development of asthma and allergic disorders.

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