

The multifunctional role of filaggrin in allergic skin disease

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Filaggrin is a major structural protein in the stratum corneum of the epidermis. Mutations in the filaggrin gene are the most significant known genetic risk factor for the development of atopic dermatitis. Mutations in the human filaggrin gene (*FLG*) also confer risk for the associated allergic diseases of food allergy, asthma, and allergic rhinitis. These discoveries have highlighted the importance of skin barrier function in the pathogenesis of atopic diseases and have motivated a surge in research characterizing the filaggrin-deficient skin barrier and its consequences. In this review we discuss the mechanisms through which mutations in this protein contribute to the pathogenesis of atopic dermatitis and associated atopic conditions. We focus on recent human and murine discoveries characterizing the filaggrin-deficient epidermis with respect to biophysical, immunologic, and microbiome abnormalities. (J Allergy Clin Immunol 2013;131:280-91.)

Key words: Filaggrin, atopy, dermatitis, eczema, IL-1 β , skin barrier, transepidermal water loss

Atopic dermatitis (AD) affects approximately 11% of children in the United States¹ and up to 25% in the United Kingdom.^{2,3} It is the most common chronic inflammatory disease of early childhood⁴ and is associated with significant morbidity for patients and their families.⁵ AD is characterized by an epidermal barrier abnormality, cutaneous inflammation, immune dysregulation with a systemic “allergic” T_H2 cell response, and frequent *Staphylococcus aureus* colonization.⁴ It is often the initial step in the so-called atopic march, with the subsequent development of allergies, asthma, and hay fever.⁶ The critical importance of the epithelium in the development of AD and allergic sensitization has become apparent. Mutations in the human filaggrin gene (*FLG*), which codes for the skin barrier protein filaggrin, have been shown to be the most significant risk factor, to date, for AD.⁴

Abbreviations used

AD:	Atopic dermatitis
AD _{FLG} :	AD with <i>FLG</i> -null mutations
AD _{NON-FLG} :	AD without <i>FLG</i> mutations
CNV:	Copy number variation
<i>FLG</i> :	Human filaggrin gene
<i>Flg</i> :	Murine filaggrin gene
<i>FLG</i> ^{+/+} :	Homozygote wild-type (ie, 0 null alleles)
<i>FLG</i> ^{+/-} :	Heterozygote null allele/wild-type (ie, 1 null allele)
<i>FLG</i> ^{-/-} :	Patient homozygous for null alleles (ie, 2 null alleles)
IsdA:	Iron-regulated surface determinant A
NLR:	Nucleotide-binding oligomerization domain–like receptor
NLRP3:	Nucleotide-binding oligomerization leucine-rich repeat and pyrin domain containing 3
NMF:	Natural moisturizing factor
OR:	Odds ratio
OVA:	Ovalbumin
PCA:	Pyrrolidone-5-carboxylic acid
SEB:	Staphylococcal enterotoxin B
SC:	Stratum corneum
SpA:	Staphylococcal protein A
TEWL:	Transepidermal water loss
TLR:	Toll-like receptor
TSLP:	Thymic stromal lymphopoietin
UCA:	Urocanic acid

The specific dynamic interactions between an impaired skin barrier and the immune system remain to be fully elucidated. Here we review recent insights into the role of filaggrin in the pathomechanisms of AD and its associated diseases.

EPIDERMAL STRUCTURE AND FUNCTION: ROLE OF FILAGGRIN

The epidermis, particularly the outermost stratum corneum (SC) layer, is the first line of defense between the host organism and its environment. Other important innate defense mechanisms of the epidermis are reviewed in detail by Dr Lisa Beck and colleagues in another review in this series. The SC also minimizes water loss from the body and protects against both everyday and extreme environmental insults.⁷ The SC is the end product of a highly organized differentiation process in which *keratinocytes* in the basal layer of the epidermis progress to form the spinous and granular layers, ultimately forming a tough multilayer of *corneocytes* rich in intracellular lipids.⁷ The SC matrix is an extensively cross-linked lipid protein matrix organized into neutral, lipid-enriched, extracellular lamellar bilayers.⁸ This hydrophobic extracellular matrix, together with corneodesmosomes and *tight*

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Terms in boldface and italics are defined in the glossary on page 281.

junctions, specialized cohesive intercellular junctions in the SC and *stratum granulosum*, forms a very effective barrier.^{9,10}

Filaggrin is a major structural protein in the SC.⁴ The role of filaggrin in epidermal structure and function has been reviewed in detail recently.^{4,7,11} Filaggrin is produced as the precursor proprotein profilaggrin. Profilaggrin is expressed in terminally differentiating keratinocytes in the outmost layers of the human epidermis and is the major constituent of keratohyalin granules in the stratum granulosum.⁷ Profilaggrin consists of multiple filaggrin repeats flanked by an S100-type calcium-binding domain, A and B domains at the N-terminus, and a unique tail sequence at the C-terminus (Fig 1, A).⁴

During terminal differentiation at the granular to cornified layer transition, profilaggrin is rapidly dephosphorylated and cleaved by several endoproteases to generate 10, 11, or 12 functional filaggrin monomers.¹² Extracellular proteases, such as matriptase, can also influence the expression of filaggrin monomers.¹³ Filaggrin monomers aggregate and align keratin bundles *in vitro* and are thus postulated to contribute to the mechanical strength and integrity of the SC *in vivo* (Fig 1, A).¹⁴ Ultrastructural studies have shown that filaggrin deficiency results in disorganized keratin filaments, impaired lamellar body loading, and abnormal architecture of the lamellar bilayer (Fig 1, B).¹⁰ It has been proposed that the filaggrin N-terminus provides a feedback mechanism that controls epidermal homeostasis.¹⁵ The exact function of the C-terminal domain is unclear, but it is necessary for profilaggrin-to-filaggrin processing. Truncated profilaggrin, lacking a C-terminus, results in an almost complete absence of filaggrin.¹²

In the upper layers of the SC, filaggrin monomers are deaminated and degraded by proteases to release their component hygroscopic amino acids and their derivatives.¹⁶ Filaggrin is a histidine-rich protein, and its major metabolites are the organic acids trans-urocanic acid (trans-UCA) and pyrrolidone-5-carboxylic acid (PCA).¹¹ Filaggrin breakdown products, together with chloride and sodium ions, lactate, and urea, form natural moisturizing factor (NMF), which contributes to epidermal hydration and barrier function (Fig 1, A).¹¹ In addition, these organic

acid breakdown products help maintain the pH gradient of the epidermis. The acidic pH is key for many functions of the SC: it has an antimicrobial effect, is important for the functional activity of enzymes involved in ceramide metabolism, and modulates the activity of the serine protease cascade required for coordinated epidermal differentiation and cornified cell envelope formation (Fig 1, B).¹¹

FLG is located in the *epidermal differentiation complex* on chromosome 1q21.⁴ Exon 3 of *FLG* is one of the largest exons in the genome and encodes almost the entire profilaggrin protein. Loss-of-function mutations within exon 3 all have a similar biological end point; they result in a truncated profilaggrin molecule lacking the C-terminus and hence an absence of filaggrin.¹² There are common size-variant *FLG* alleles in the general population, with 10, 11, or 12 repeats.¹⁷ Therefore the number of filaggrin units in human subjects varies from 20 to 24, excluding null mutations. The frequencies of copy number variation (CNV) alleles have been studied in the Irish population; 33.9% had 10 filaggrin repeats, 51.1% had 11 repeats, and 14.6% had 12 repeats.¹⁷

FILAGGRIN: DISEASE ASSOCIATIONS

In 2006, *FLG* mutations were shown to be strongly associated with AD in an Irish population and with AD plus asthma in a Scottish population.¹⁸ This highly significant association has been replicated in more than 30 independent studies.¹¹ Meta-analyses of these data have estimated the odds ratio (OR) of having AD in association with an *FLG*-null genotype to be 4.78¹⁹ and 3.12.²⁰

FLG-null mutations are seen in less than a third of the total population with AD.^{18,21} Among patients with moderate-to-severe AD, up to 45.7% to 56.6% carry 1 or more *FLG*-null mutations, and the population attributable risk fraction has been estimated at between 4.2% and 15.1%.²² Therefore on a population level, approximately 50% of moderate-to-severe AD cases can be attributed, at least in part, to *FLG*-null mutations, whereas up to 15% of mild-to-moderate AD cases can be explained by *FLG*.²² Among a group of patients with AD attending a tertiary referral clinic,

GLOSSARY

ANTIMICROBIAL PEPTIDES: Components of the innate immune system that are capable of inserting into bacterial phospholipids to slow microbial growth.

CORNEOCYTES: Terminally differentiated anucleated cells on the surface of the skin. Because they are rich in intracellular lipids, they are able to bind 3 times their weight in water.

EPIDERMAL DIFFERENTIATION COMPLEX: A cluster of approximately 60 genes located on chromosome 1q21 involved in epithelial differentiation.

HAPLOINSUFFICIENCY: The situation in which a subject who is heterozygous for a certain gene mutation or hemizygous at a particular locus, often because of a deletion of the corresponding allele, is clinically affected because a single copy of the normal gene is incapable of providing sufficient protein production to assure normal function.

KERATINOCYTES: A structurally important and immunologically active epidermal cell that undergoes a tightly regulated terminal differentiation program from stem cells in the basal cell layer through the spinous and granular layers to eventual desquamation of the stratum corneum.

OVALBUMIN: The most abundant egg white protein (Gal d 2 allergen nomenclature). Although ovalbumin comprises the majority of egg

white protein, ovomucoid (Gal d 1) is responsible for the majority of allergy in patients with egg allergy.

PATHOGEN RECOGNITION RECEPTORS: Also known as pattern recognition receptors. They are germline encoded and expressed constitutively by both immune and nonimmune cells. They recognize conserved microbial components known as pathogen-associated molecular patterns.

STRATUM GRANULOSUM: An outer layer of the epidermis immediately proximal to the stratum corneum. Keratinocytes in this layer are flattened, and their cytoplasm appears granular.

SUPERANTIGEN: A class of T-cell receptor ligands that have the ability to activate large fractions of the T-cell population. They do not require processing to bind to MHC class II molecules. They bind to polymorphic residues on the periphery of the class II molecule and interact with the T-cell receptor through their V β domain.

TIGHT JUNCTION: A dynamic network of proteins that help seal the apical space between epithelial cells and regulate epithelial permeability. Proteins that are found in the tight junction can include junctional adhesion molecule 1, occludin, and claudins. The cysteine and serine protease components of the house dust mite allergen (Der p 1) can cleave junctional proteins.

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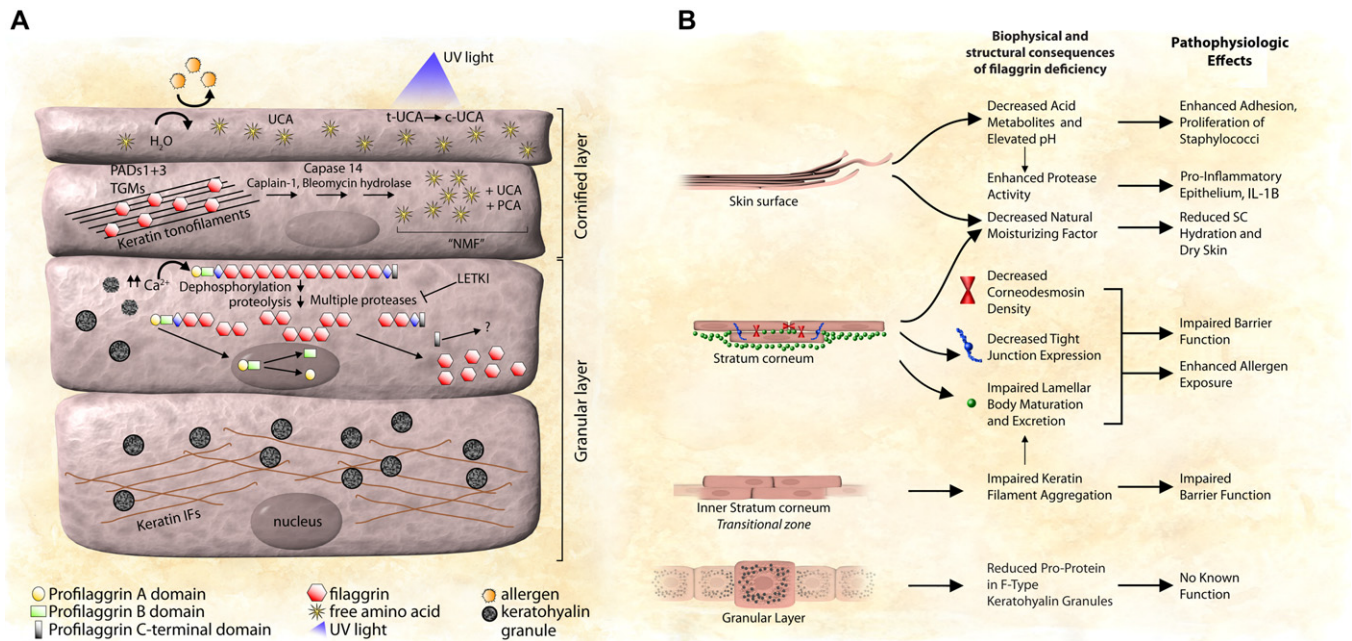


FIG 1. The role of filaggrin in the skin and the structural and biophysical consequences of filaggrin deficiency. **A**, The SC is produced by a highly organized differentiation process in which keratinocytes in the basal layer of the epidermis move through the spinous and granular layers.⁷ Profilaggrin is the major constituent of keratohyalin granules in the stratum granulosum and is expressed in terminally differentiating keratinocytes in the outermost layers of the human epidermis. Profilaggrin consists of multiple copies of filaggrin flanked by an S100-type calcium-binding domain, A and B domains at the N-terminus, and a unique tail sequence at the C-terminus. Terminal epidermal differentiation is calcium dependent, and calcium might be involved in the control of profilaggrin processing. Inactivation of lymphoepithelial Kazal type-related inhibitor (*LETKI*) is necessary to allow processing to be initiated. During terminal differentiation at the granular to cornified cell transition, profilaggrin is dephosphorylated and cleaved by several proteases, including caspase-14, matrilysin, prostasin, kallikrein 5, and elastase-2, to functional filaggrin monomers. Skin-specific retroviral-like aspartic protease (SASPase) is required for cleaving individual filaggrin monomers. Filaggrin monomers are thought to aggregate and align keratin bundles in the cornified cell envelope and are thus postulated to contribute to compaction of squames and thus to the mechanical strength and integrity of the SC. The free N-terminal domain of profilaggrin has been shown to translocate into the nucleus, where it might have a signaling function. The function of the C-terminus is unknown, but its absence because of truncation mutations leads to profilaggrin degradation. In the upper (cornified) layers of the SC, filaggrin monomers are deiminated and degraded by proteases to release their component hygroscopic amino acids. Peptidylarginine deiminase (*PAD*) isoforms 1 and 3 are involved in the deimination process. The major metabolites are the organic acids trans-UCA and PCA. Filaggrin breakdown products form NMF, which contributes to epidermal hydration and barrier function; help maintain the pH gradient of the epidermis, which is key for many functions of the SC; and possibly plays a role in UV protection. *TGMs*, Transglutaminases. **B**, The filaggrin-deficient skin barrier has reduced proprotein in F-type keratohyalin granules. The consequences of this are as yet unknown. Ultrastructurally, *FLG* loss-of-function mutations are associated with disorganized keratin filaments, impaired lamellar body loading, and abnormal architecture of the lamellar bilayer. There is also reduction in corneodesmosome density and tight junction expression. These factors might contribute to the dysfunctional skin barrier and enhanced allergen exposure. *FLG*-null mutations also result in decreased levels of NMF, reduced SC hydration, and increased TEWL and clinically dry skin. The acidic pH of the SC is key for many functions; it has an antimicrobial effect, is important for the functional activity of enzymes involved in ceramide metabolism, and modulates the activity of the serine protease cascade required for coordinated epidermal differentiation and cornified cell envelope formation. The reduction in filaggrin breakdown amino acids causes an increase in SC pH. This more alkaline pH enhances protease activity and might contribute to the proinflammatory SC in the setting of AD, as well as facilitating adhesion and proliferation of staphylococci.

3% were homozygous for the *FLG*-null genotype, whereas 20% were heterozygous.²³ A study of an unselected population cohort of children demonstrated that the penetrance of *FLG*-null mutations with respect to flexural AD was 55.6% for homozygous and compound heterozygous subjects compared with 16.3% for heterozygous subjects.²⁴ Patients who were *FLG*-null homozygous had statistically significantly higher severity scores than heterozygous and wild-type patients.²⁴

The profile of AD most strongly associated with *FLG*-null mutations (*AD_{FLG}*) is that of early-onset, severe, and persistent disease^{25,26} with increased total IgE levels and allergic sensitization.²⁷ Furthermore, patients with *AD_{FLG}* have a higher incidence of skin infections with herpesvirus (eczema herpeticum),²⁸ as well as a greater risk of multiple allergies¹⁹ and asthma,²⁹ than patients with AD without *FLG* mutations (*AD_{NON-FLG}*). A US longitudinal cohort study suggested that there might be mutation-specific

variability in the response to treatment in children with AD_{FLG}.²⁶ Taken together, these studies suggest that patients with AD_{FLG} might have a distinct AD endophenotype and profile of associated disease compared with patients with AD_{NON-FLG} (Fig 2).⁴

It has been demonstrated that AD risk in an Irish population is related to filaggrin CNV in a dose-dependent fashion.¹⁷ The lowest CNV genotype (10, 10 filaggrin repeats) carried by 11.5% of the Irish population had an eczema risk of 1.67 independently of *FLG* loss-of-functions mutations. The addition of each additional filaggrin repeat decreased the OR for AD by 0.88.¹⁷ Furthermore, the concentration of filaggrin breakdown products was significantly correlated with filaggrin total CNV.¹⁷ Thus a modest increase in epidermal filaggrin expression might be protective against eczema, and upregulation of cutaneous filaggrin expression in at-risk subjects might be a potential therapeutic approach (Fig 3).¹¹ Recent work suggests that the methylation status of *FLG* might further influence AD risk.³⁰

The strong association of *FLG* mutations with AD is one of the most robust genotype-phenotype linkages observed in complex human genetic disorders.¹⁹ However, pathomechanisms other than *FLG* mutations or *FLG*-modifying factors are involved in AD. A significant number of patients with AD do not have any of the known *FLG* mutations, and conversely, approximately 40% of subjects with *FLG*-null alleles do not have AD.³¹ In addition, many patients with AD_{FLG} eventually recover from the disease.²⁹ More work is needed to establish influences other than *FLG* on epidermal barrier defects in patients with AD.

The “atopic march” describes the tendency for AD to precede the stepwise development of food allergies, asthma, and allergic rhinitis. Approximately 70% of patients with severe AD will have asthma or allergic rhinitis in later life.⁶ *FLG* mutations are a genetic risk factor for each of these diseases.⁴ Filaggrin *haploinsufficiency* confers an overall risk of 1.48 to 1.79 for asthma, but this risk is limited to those who have AD or a history of the disease.^{29,32} Patients with AD_{FLG} have a much greater risk of asthma than patients with AD_{NON-FLG}.²⁹ Asthmatic patients with *FLG* mutations have a more difficult disease course and more frequent exacerbations.³³ Therefore AD is a causal risk factor for asthma in the context of *FLG* mutation, although the mechanism is not fully elucidated.⁴

FLG mutations confer an overall OR of 5.3 for peanut allergy, with a residual OR of 3.8 when corrected for AD.³⁴ These data suggest a barrier defect that facilitates enhanced exposure of peanut allergen to antigen-presenting cells, even in the absence of AD. An Australian cohort study found that *FLG* mutations were significantly associated with food sensitization but did not additionally increase the risk of food allergy in 1-year-old infants.³⁵ These results suggest that the skin barrier dysfunction increases the risk of food sensitization, but other factors might be important in the conversion from food sensitization to allergy.³⁵ An association with *FLG* and allergic rhinitis has been reported in population studies.^{19,32}

Filaggrin immunostaining is restricted to the cornified epithelium of the skin, oral mucosa, and nasal vestibule. There is no detectable staining in the epithelium from bronchial biopsy specimens.^{32,36,37} Therefore *FLG* mutations are unlikely to directly affect barrier function and allergen sensitization in the organs in which these allergic diseases manifest. It is possible that *FLG* mutations drive allergic disease at distant mucosal sites as a systemic response to enhanced penetration by antigens through a defective skin barrier.³⁸

AD _{FLG}	Clinical Features	Biophysical Features
	Palmar Hyperlinearity	Severe Decrease in Natural Moisturizing Factor (NMF)
	More Persistent	↑pH
	↑Allergic Sensitization	↑IL-1β
	↑Risk of Asthma	
	↑Severity	
	↑Eczema Herpeticum	
AD _{NON-FLG}	Clinical Features	Biophysical Features
	No Palmar Hyperlinearity	Mild Decrease in Natural Moisturizing Factor (NMF)
	Less Persistent	pH Lower Compared to AD _{FLG}
	Less Allergic Sensitization	IL-1β Low Compared to AD _{FLG}
	Lower Risk of Asthma	

FIG 2. Comparison of the clinical and biophysical features of patients with AD_{FLG} and patients with AD_{NON-FLG}. Patients with AD_{FLG} have a particular AD endophenotype or profile of associated disease and biophysical features. Patients with AD_{FLG} have palmar hyperlinearity, which is also observed in patients with ichthyosis vulgaris, the Mendelian disease caused by *FLG* mutations. Patients with AD_{FLG} have more severe persistent eczema and a higher incidence of infection with herpesvirus, as well as a greater risk of allergic sensitization and asthma than patients with AD_{NON-FLG}. The biophysical profile of AD_{FLG} shows an increased SC pH and production of IL-1β in patients with AD_{FLG} compared with that seen in patients with AD_{NON-FLG}.

FILAGGRIN: ENVIRONMENTAL INTERACTIONS

The prevalence of AD has more than doubled in industrialized countries, with no clear cause.^{1,39} Environmental factors are thought to contribute to this increasing prevalence. It has been postulated that the impaired skin barrier seen with *FLG* might potentiate the effects of environmental allergens.⁴⁰ Some studies have investigated putative environmental risk factors for atopic disease genotype with regard to *FLG* status.

Two cohort studies, one from Denmark and the United Kingdom and the other from The Netherlands, have shown that cat ownership in early life increases the risk of AD as an additional interactive effect to the risk associated with the *FLG*-null genotype.^{40,41} However, the Danish–United Kingdom study did not demonstrate any correlation between AD severity and specific IgE to cat dander or *FLG*-null mutations and cat dander IgE levels,⁴⁰ making the mechanism of this association likely to be a host-defense interaction between the microbiome and a defective skin barrier.

Another potentially important environmental effect in early life is contact with other children because this can increase exposure to pathogens and allergens. Two German birth cohort studies have shown that children with *FLG*-null mutations have a significantly higher risk of eczema if they have an older sibling and that attendance at a day care center lessened this risk, reducing the OR from 2.34 to 1.7.⁴² Epidemiologic data can be difficult to interpret in a complex disease such as AD. Different causal pathways between genes and the environment might be important in patients with AD who carry mutations, as opposed to those who do not. It would be important, where possible, to stratify for *FLG* in future epidemiologic studies of AD.¹¹

The significant discovery that *FLG* mutations are a strong risk factor for AD and atopic diseases have confirmed the key role of the skin barrier in these conditions. The result has been a research emphasis on functionally characterizing the skin barrier, as well as

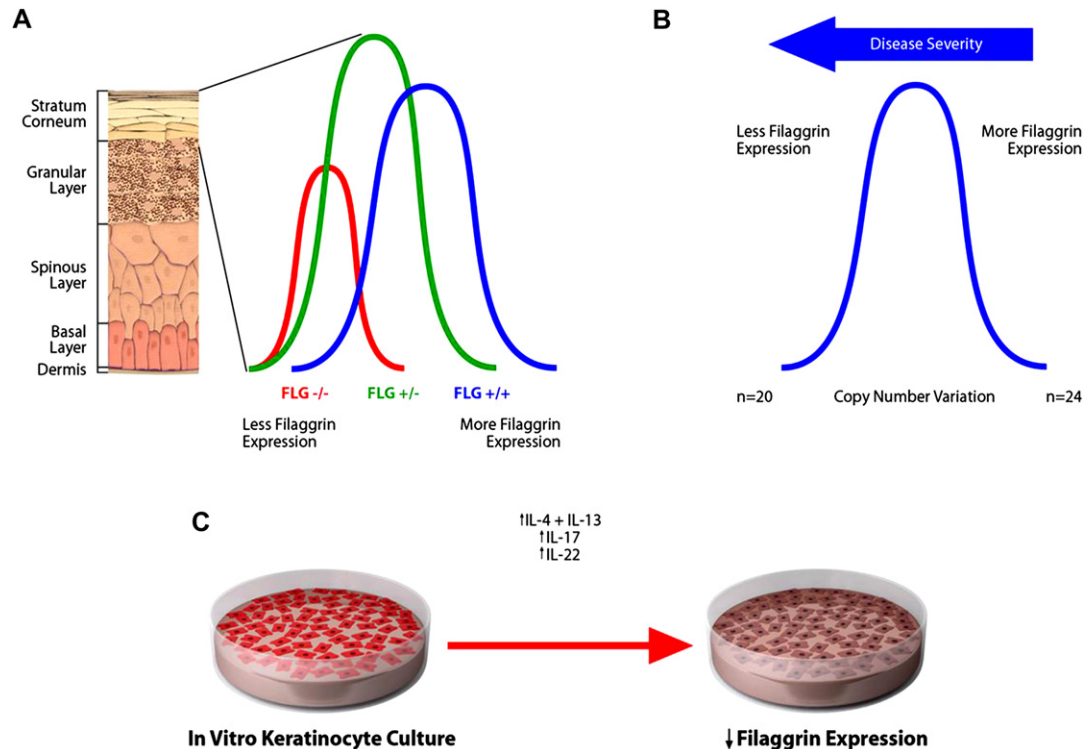


FIG 3. Known genetic and immunologic influences on filaggrin expression. **A**, The major determinant of filaggrin expression is *FLG* genotype, with 3 distinct but overlapping populations according to the number of *FLG* loss-of-function mutations. The observed interindividual variation in NMF within the mutation groups approximates to a normal distribution curve, which reflects additional genetic and environmental modifiers of filaggrin expression. **B**, There are common size-variant *FLG* alleles in the population with 10, 11, or 12 repeats. Excluding null mutations, the number of filaggrin units in human subjects, termed the filaggrin CNV, varies from 20 to 24. AD risk is related to filaggrin CNV. In keeping with this, the concentration of filaggrin breakdown products (NMF), as quantified by means of HPLC of tape-stripped SC, is statistically significantly correlated to filaggrin CNV. Furthermore, disease severity drives down filaggrin expression independently of *FLG* mutation status. **C**, Filaggrin expression *in vitro* is downregulated in the presence of inflammatory cytokines. Keratinocyte cultures differentiated in the presence of IL-4 and IL-13 exhibit significantly reduced filaggrin gene expression. Exposure to IL-22 cytokine downregulates profilaggrin/filaggrin expression in keratinocytes at both mRNA and protein level. Keratinocytes cultured with IL-17A also resulted in a significant decrease in profilaggrin mRNA levels and filaggrin protein expression. IL-17A appears to downregulate filaggrin expression at the mRNA level both directly and indirectly. Patients with AD_{FLG} and AD_{NON-FLG} have an acquired defect in filaggrin secondary to the presence of inflammatory cytokines. In the setting of AD_{FLG}, the combination of genetically determined and acquired filaggrin insufficiency can lead to a greater and more prolonged filaggrin downregulation.

identifying pathways connecting epidermal barrier disruption, antigen uptake, and the antigen-specific adaptive immune responses.

CHARACTERIZATION OF THE FILAGGRIN-DEFICIENT SKIN BARRIER

The barrier integrity phenotype associated with *FLG* mutations is becoming better understood, with human and murine studies supporting the theory that *FLG* mutations lead to a functional epidermal barrier defect and subsequent allergic sensitization.

FLG genotype has been shown to be the major determinant of SC NMF in human studies. The SC levels of the filaggrin breakdown products PCA, UCA, and histidine, which are major components of NMF, in epidermal tape strips, strongly correlate with *FLG* genotype.⁴³ O'Regan et al⁴⁴ demonstrated that *in vivo* Raman microspectroscopic NMF signatures could be used as accurate proxy markers of *FLG* genotype in patients with moderate-to-severe AD, allowing rapid and highly accurate stratification of AD_{FLG} (Fig 3). However, AD severity itself is associated with a

reduction in NMF, and the relative importance of epidermal defects and immune dysregulation as key initiating and perpetuating factors in AD pathogenesis require further studies.¹¹

Transepidermal water loss (TEWL) at nonlesional sites in patients with AD correlates with disease severity and serum IgE levels.⁴⁵⁻⁴⁷ Several studies suggest that nonlesional TEWL in patients with AD is a common end point that is not influenced by *FLG* status.^{44,48,49} However, *FLG* mutations were associated with higher TEWL in clinically normal forearms in a small cohort of 3-month-old infants in a high-risk cohort; this increase was not dependent on AD status.⁵⁰ Further studies are needed to clarify the temporal and causal relationship between TEWL, barrier integrity, *FLG* status, and subsequent allergen sensitization.³⁸

MECHANISTIC INSIGHTS FROM MURINE MODELS

Mouse models of filaggrin deficiency have demonstrated barrier impairment with enhanced percutaneous allergen

sensitization.⁵¹⁻⁵⁴ The spontaneous flaky tail (*ft*) mouse arose on the background of an existing recessive hair phenotype, matted (*ma*). Flaky tail mice carry a 1-bp deletional mutation in the murine filaggrin gene (*Flg*). The relative contribution of *Flg* and *ma* to the compound phenotype has yet to be fully defined. Flaky tail mice have spontaneous dermatitis with increased IgE levels.⁵⁴ Fallon et al⁵¹ demonstrated that the topical application of the clinically relevant allergen *ovalbumin* (OVA) to flaky tail (*ft/ft*) mice resulted in cutaneous inflammation and enhanced cutaneous allergen priming with development of allergen-specific antibody responses. The mice had a systemic immune response generating OVA-specific IgG and IgE, as well as OVA-specific T_H2 (IL-4, IL-5, and IL-13), T_H1 (INF- γ), regulatory (IL-10), and T_H17 (IL-17) cytokines, indicating a generalized allergen-specific cytokine response that was not solely T_H2 skewed. After sensitization, a further skin barrier defect, as measured by increased TEWL, was observed, suggesting that the initial heritable barrier defect is exacerbated by allergic sensitization. These data provide experimental evidence that antigen transfer through a defective epidermal barrier is a key mechanism underlying increased IgE sensitization and initiation of cutaneous inflammation. This suggests that sensitization might also be an early event in filaggrin-deficient human subjects.⁵¹ Whether early intervention in patients with AD, especially filaggrin-deficient AD, would diminish systemic allergy in later life is an interesting research question.

Kawasaki et al⁵² generated filaggrin-null mice (*Flg*^{-/-}).⁵² These mice have dry scaly skin between 3 and 6 days of life. They have loss of the normal interlaced keratin pattern in the epidermis with increased susceptibility to mechanical stress. *In vivo* confocal microscopy showed reduced NMF levels in the *Flg*^{-/-} mice,⁵² which is in keeping with human studies on patients with *FLG* mutations.⁴⁴ The loss of NMF as a result of filaggrin deficiency did not lead to decreased SC water content in the *Flg*^{-/-} mice. This is in contrast to the *ft/ma* mice, which have increased TEWL with loss of SC hydration, which is consistent with findings with human AD (with and without *FLG* mutations).⁴⁹ *Flg*^{-/-} murine SC, after hapten application, allowed penetration of protein antigens, which was followed by exaggerated systemic immune responses.⁵² It is notable that the SC lipid composition in the *Flg*^{-/-} mice was aberrant, and this might have additional direct or indirect effects on SC barrier function.⁵²

These murine studies support the hypothesis that filaggrin deficiency results in enhanced percutaneous cellular and humoral immune responses, which are important steps in the early phase of AD pathogenesis. This important work characterizing a dysfunctional skin barrier and downstream systemic effects has provided an opportunity to focus on the importance of barrier improvement as a key therapeutic approach in this disease. Tailored emollients, such as ceramide lipid,⁵⁵ or filaggrin replacement or upregulation are exciting possibilities.¹¹ In addition, these studies support the notion that a proactive rather than reactive approach to eczema management could have a positive effect on systemic sensitization and the atopic march. Perhaps a prophylactic approach might be possible, with regular use of emollients or other topical therapies, immediately after birth in high-risk babies, reducing the risk of AD. Large, well-designed, randomized controlled trials will be needed to answer these intriguing questions.

FILAGGRIN STATUS AND IMMUNE DYSREGULATION: A COMPLEX INTERACTION

Both innate and adaptive immunity contribute to the immunopathology of AD. Innate responses occur rapidly, are efficient at killing pathogens, and are involved in regulating the magnitude and specific outcomes of the adaptive immune response.⁵⁶ The cutaneous innate immune system consists of 3 major components: the physical barrier, which includes the SC and intracellular junctions; the cellular component (antigen-presenting cells, keratinocytes, mast cells, and neutrophils); and secretory elements (*antimicrobial peptides*, cytokines, and chemokines), as reviewed in detail by De Benedetto et al.⁵⁶ In patients with AD, the initial exposure to allergens (sensitization phase) induces a systemic "allergic" T_H2 cell response that is magnified with each subsequent exposure (effector phase). Critical features of the T_H2 immune response include local production of T_H2 cytokines (IL-4, IL-5, and IL-13), bone marrow production, prolonged survival and activation of eosinophils and mast cells, and production of allergen-specific IgE.³⁸ Acute AD lesions exhibit T_H2-dominant inflammation characterized by dermal infiltration of CD4⁺ T cells and eosinophils with deposition of eosinophil-derived products and increased skin expression of IL-4, IL-5, and IL-13.⁵⁷ A pathogenic role for IL-4 in the setting of AD is supported by the observation that keratinocyte-specific overexpression of IL-4 in transgenic mice results in AD-like lesions.⁵⁸ Subjects with *FLG*-null mutations have been associated with significantly higher frequencies of allergen-specific CD4⁺ T_H2 cell responses.⁵⁹

Filaggrin expression is downregulated in patients with AD, regardless of *FLG* genotype, likely because of the effect of increased levels of the T_H2 cytokines IL-4 and IL-13 (Fig 3).⁶⁰ Keratinocytes differentiated in the presence of IL-4 and IL-13, as well as already differentiated keratinocytes exposed to these cytokines, have significantly downregulated filaggrin expression.⁶⁰ These findings support the theory that filaggrin deficiency in many patients with AD is acquired due to the T_H2 cytokine milieu.⁶⁰

The specific pathways linking epidermal barrier disruption and allergen sensitization are becoming clearer. One accepted hypothesis is that epidermal disruption facilitates skin-resident antigen-presenting cells (Langerhans and dendritic cells) in capturing environmental allergens because of either more direct exposure through a defective SC or direct effects on Langerhans cell activation. Furthermore, barrier-disrupted keratinocytes release immune adjuvants that activate and cause maturation of antigen-presenting cells and affect their ability to direct native T_H polarization, thereby influencing the character of the T_H response. The resulting adaptive immune response further disrupts barrier function.³⁸

The cytokine profile in the epidermis in patients with AD_{FLG} is now becoming clearer. However, the majority of studies to date are *in vitro* or in murine models. One study has investigated SC cytokines from patients with AD stratified by *FLG* status.⁶¹ Here we review recent data examining the interaction between filaggrin status and the immune response.

IL-1

IL-1 mediators influence innate immune responses and bridge the innate and adaptive immune systems. Keratinocytes constitutively produce high amounts of IL-1 α .^{62,63} In inflammatory states human keratinocytes also produce IL-1 β .⁶⁴ The release of

IL-1 cytokines leads to cutaneous inflammation through the induction of secondary cytokines and the upregulation of endothelial adhesion molecules.^{62,65} The multiple proteases necessary for epidermal homeostasis and cleavage of IL-1 cytokines have optimal activity at pH values that are more alkaline than the SC surface.⁶⁶

Murine studies have indicated the importance of IL-1 β and IL-18 for the development of AD. Skin-specific caspase-1–transgenic mice, which overexpress human CASP1 in their keratinocytes, spontaneously had chronic dermatitis accompanied by abnormally increased skin and serum IL-18 and IL-1 β levels when maintained under pathogen-free conditions.⁶⁷ An IL-18 transgenic mouse that exhibited oversecretion of IL-18 from epidermal cells had AD-like skin eruptions. This phenotype was rescued by knockout of IL-18.⁶⁸ Furthermore, an AD mouse model generated through the daily application of protein A (*Staphylococcus aureus* surface model and virulence factor) resulted in complete amelioration of the AD-like skin eruptions through either administration of a neutralizing anti-IL-18 antibody or IL-18 gene knockout.⁶⁹

SC IL-1 α , IL-1 β , IL-18, and IL-1 receptor antagonist levels were recently shown to be increased in the uninvolved skin of patients with moderate-to-severe AD_{FLG} compared with that seen in patients with AD_{NON-FLG}.⁶¹ IL-1 cytokine levels were correlated inversely with SC NMF levels. An association was demonstrated between increased pH and decreased NMF levels. Although AD severity influences SC NMF, this was shown to be a minor effect compared with *FLG* status, which was the major determinant of NMF (Fig 1, B).⁶¹ These findings were also observed in a complementary murine study. Filaggrin-deficient mice (*fl/fl*) had upregulated expression of IL-1 β and IL-1 receptor antagonist in the SC.⁶¹

Thus it is possible that a reduction in filaggrin and its acidic breakdown products increases pH and serine protease activity, contributing to the generation of the active cytokines IL-1 α and IL-1 β from their inactive proproteins, representing the first stage of the cytokine cascade that contributes to AD inflammation.⁶¹ These cytokines have a further inhibitory effect on *FLG* expression. This work suggests that there might be a pre-existing or enhanced proinflammatory status in the skin of patients with AD_{FLG} (Fig 1, B).⁶¹

The inflammasome

The work on IL-1 in the setting of AD_{FLG} is consistent with prior studies on the inflammasome in atopy. The innate immune system senses invading pathogens through evolutionarily conserved **pathogen recognition receptors**, such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain–like receptors (NLRs). NLR members form an intracellular multiprotein complex, the inflammasome.⁷⁰ Inflammasomes enable autocatalytic activation of inflammatory caspases, which drive the host immune response by releasing cytokines and alarmins into the circulation and by inducing pyroptosis, a proinflammatory cell death mode.⁷¹ The inflammasome activates caspase-1 and ultimately leads to the processing and release of the proinflammatory cytokines IL-1 β , IL-18, and IL-33.⁷⁰ There is strong evidence that inflammasomes play an important role in skin inflammation.⁷²

Research has focused on the nucleotide-binding oligomerization leucine-rich repeat and pyrin domain containing 3 (NLRP3) inflammasome, which is made up of NLRP3, an apoptosis-associated speck-like protein containing a caspase recruitment

domain, and caspase-1.⁷⁰ An association has been reported between the NLRP3 inflammasome and susceptibility to food-induced anaphylaxis and aspirin-induced asthma.⁷³ Hemolysins and bacterial lipoproteins from *S aureus* can activate the NLRP3 inflammasome.^{74,75} It has been recently shown that house dust mite allergens trigger assembly of the NLRP3 inflammasome, activate caspase-1, and thus stimulate the processing and release of IL-1 β and IL-18 from keratinocytes *in vitro*.⁷⁶ The release of these cytokines might trigger or exacerbate AD-associated inflammation and be important in the pathogenesis of the disease.⁷⁶

Thymic stromal lymphopoietin

Thymic stromal lymphopoietin (TSLP) is an IL-7–like cytokine that has a key role in T_H2 cell differentiation and in the pathogenesis of allergic inflammation.⁷⁷ TSLP is highly expressed in the epidermis of patients with AD, and TSLP-activated dendritic cells produced T_H2-attracting chemokines and primed naive T cells to differentiate into T_H2 cells.⁷⁸ An increased serum TSLP level is an indicator of an epidermal barrier defect in mouse models.⁷⁹ Proteases, through proteinase-activated receptor 2, can induce TSLP expression from keratinocytes or airway epithelial cells.^{80,81} A number of allergens that are clinically relevant in AD, including house dust mite, cockroach, fungi, and several pollens, contain proteases that can trigger epithelial production of TSLP *in vitro*.⁸² Epithelial TSLP is also induced through a TLR2-, TLR3-, and TLR5-mediated mechanism in response to microbial products.³⁸ TSLP expression in a reconstituted human epidermal layer was increased under filaggrin knockdown conditions.⁸³ This work suggests that filaggrin deficiency induces TSLP expression and a resultant T_H2 immune reaction.⁸³ TSLP might be more important in the elicitation phase rather than the sensitization phase of AD.^{38,84}

IL-33

IL-33 is a novel member of the IL-1 family and is expressed by the cells of barrier tissues. It is recognized as an alarmin or damage-associated molecular pattern molecule because it is released during epithelial cell death, is associated with infection or tissue injury, and is induced by microbial ligands through a TLR-mediated pathway.⁸⁵ IL-33 activates naive and T_H2 lymphocytes, mast cells, and eosinophils to produce T_H2-type cytokines. Furthermore, mast cells produce IL-33 in response to IgE-dependant activation, and IL-33 amplifies the inflammation resulting from mast cell and basophil activation.^{86,87}

IL-33 levels are markedly increased in the serum of patients with asthma and in the skin of patients with AD.⁸⁸ Increased levels of IL-33 and its specific receptor, ST2, have been demonstrated in AD skin after allergen or staphylococcal enterotoxin B (SEB) exposure, as well as in the skin of filaggrin-deficient mice.⁸⁹ The expression of IL-33 and ST2 were spontaneously upregulated in the skin of 22-week-old *fl/fl* mice, and a 10-fold and a 15-fold increase in ST2 and IL33 expression was found, respectively, in 38-week-old *fl/fl* mice compared with 4-week-old *fl/fl* mice. The expression of IL-33 caused by irritant, allergen, or SEB challenge was suppressed in flaky tail (*fl/fl*) mouse skin by topical tacrolimus treatment.⁸⁹ This work suggests that keratinocytes of *fl/fl* mice respond to environmental factors and start to produce cytokines related to innate immunity.⁸⁹

IL-25

IL-25 (or IL-17E) is a member of the IL-17 cytokine family that, when overexpressed in murine models, results in the production of T_H2 cytokines, eosinophilia, and increased serum IgE levels.⁹⁰ IL-25 is expressed by mouse epithelial cells after allergen stimulation⁹¹ and in the human skin of patients with AD.⁹² It has been shown that IL-25 inhibits filaggrin synthesis by keratinocytes, and therefore IL-25 might contribute to barrier dysfunction in patients with AD.⁹³

IL-22

IL-22 is another cytokine that could play a critical role in AD. T_H22 cells are a skin-homing phenotype and are a potent source of IL-22.⁹⁴ Numbers of T_H22 , T_C22 , and T_H17 cells, which secrete IL-22, are increased in the peripheral blood of patients with AD and are observed in inflamed skin.⁹⁵⁻⁹⁸ In addition, cutaneous dendritic cells have been shown to induce a T_H22 phenotype in T cells.⁹⁹ IL-22 secretion by PBMCs and $CD4^+$ T cells is enhanced by *S aureus* exotoxins in patients with AD.¹⁰⁰ Exposure to IL-22 downregulated profilaggrin/filaggrin expression in keratinocytes *in vitro* at both the mRNA and protein levels. An alteration in the expression of genes encoding enzymes involved in profilaggrin/filaggrin processing was also observed, suggesting that IL-22 could also affect pathways generating functional filaggrin monomers.¹⁰¹

IL-17

IL-17 is another important cytokine in the setting of AD. Serum and skin levels of IL-17 are increased in patients with AD compared with healthy control subjects, and T_H17 cells were found to accumulate at early stages of skin inflammation in patients with AD.^{98,102} Furthermore, IL-17 is chronically present during skin inflammation, especially when exposed to *S aureus* or allergens.^{102,103} The filaggrin-deficient flaky tail mouse exhibits T_H17 -dominated skin inflammation from an early age, even before increased IL-4 expression.⁵⁴

Recent work has shown that stimulation of keratinocyte cultures with IL-17A results in a significant decrease in profilaggrin mRNA levels and filaggrin protein expression (Fig 2).¹⁰⁴ Several genes encoding proteins were affected by IL-17, suggesting that IL-17A downregulates filaggrin expression at the mRNA level both directly and indirectly by affecting profilaggrin mRNA expression, production of functional filaggrin monomers, and their degradation.¹⁰⁴ IL-17A appears not only to influence filaggrin expression but also to affect the expression of other important components of the epidermal barrier.¹⁰⁴

Evidence is mounting that filaggrin deficiency plays an important role in the cytokine profile of patients with AD. Cytokines have a further inhibitory effect on filaggrin expression, and thus a feedback loop probably exists in this setting. The cytokines involved in the pathogenesis of AD, similar to other inflammatory skin diseases, are multiple and complex, and much work will be needed to clarify these pathways. As yet, there is no effective and specific biologic treatment for AD. A greater understanding of such functional mechanisms involved in the disease is needed to identify potential therapeutic targets.

FILAGGRIN DEFICIENCY AND THE MICROBIOME

The skin microbiome, which consists of both commensal and pathogenic bacteria, affects the skin barrier and epithelial innate immune responses. Skin microbes are thought to have a critical role in the development of AD. Patients with AD experience frequent bacterial and viral cutaneous infections. More than 90% of patients with atopic eczema are colonized with *S aureus* in comparison with 5% of healthy subjects.¹⁰⁵ The severity of dermatitis correlates with both colony counts of *S aureus* colonized from AD skin¹⁰⁶ and the presence of *superantigen*-producing *S aureus*.^{107,108}

Bacteria have to overcome acidic conditions, antimicrobial peptides, and fatty acids to survive on skin.¹⁰⁹ *S aureus* colonization in patients with AD is promoted by host and microbial mechanisms, including the dysfunctional skin barrier and bacterial surface-associated proteins that can bind to host adhesive molecules.¹⁰⁹ *S aureus* surface-associated proteins and virulence factors also contribute to inflammation.¹⁰⁹ Furthermore, high levels of T_H2 cytokines inhibit cutaneous antimicrobial peptides, further promoting bacterial proliferation.¹¹⁰ The surface protein staphylococcal protein A (SpA) stimulates cytokine release and subsequent inflammation on airway epithelial cells.¹¹¹ In combination with subclinical levels of detergent, SpA has been demonstrated to induce skin inflammation in animal models.⁶⁹

Miajlovic et al¹⁰⁹ investigated *S aureus* in the presence of UCA and PCA. These filaggrin breakdown products, at physiologic concentrations, demonstrated an inhibitory effect on the growth of *S aureus*.¹⁰⁹ Therefore the increase in SC pH in patients with AD might lead to enhanced *S aureus* adhesion and multiplication.¹⁰⁹ Furthermore, there was a decreased expression of iron-regulated surface determinant A (IsdA) in the presence of these filaggrin breakdown products that was independent of pH. Therefore UCA and PCA appear to have a specific antistaphylococcal effect by directly inhibiting this surface protein. IsdA promotes bacterial adhesion to squames and plays a role in *S aureus* survival on the skin.¹⁰⁹ Thus a reduction in filaggrin breakdown products in patients with AD, either from *FLG*-null alleles or from T_H2 inflammation, might increase expression of staphylococcal IsdA and promote survival of *S aureus* (Fig 4).¹⁰⁹ Therapies that reduced the SC pH could positively affect disease severity by minimizing *S aureus* colonization and improving epidermal function.¹⁰⁹ Application of low-pH creams and acidic electrolytic water on epithelial surfaces has been shown to reduce *S aureus* colonization in AD.^{112,113}

Approximately 50% of *S aureus* isolates from patients with AD produce superantigens, including SEB.¹¹⁴ The ability of superantigens to cause stimulation of T cells and macrophages, Langerhans cells, and activated keratinocytes accounts for the majority of their pathological effect.¹¹⁵ Superantigen production by *S aureus* strains is positively correlated with T-cell activation and increased severity of disease in patients with AD.¹¹⁶ In addition, staphylococcal superantigens induce the production of superantigen-specific IgE in patients with AD.¹¹⁷ Sensitization to superantigen-specific IgE has been correlated with AD severity.¹¹⁸

SEB is shown to enhance house dust mite-induced patch test reactions in patients with AD.¹¹⁹ Topical SEB superantigen exposure in the skin induces a mixed T_H1/T_H2 -type dermatitis and production of IgE antibodies in a murine model of AD in wild-type mice.¹²⁰ Epicutaneous exposure of superantigen SEB in mice

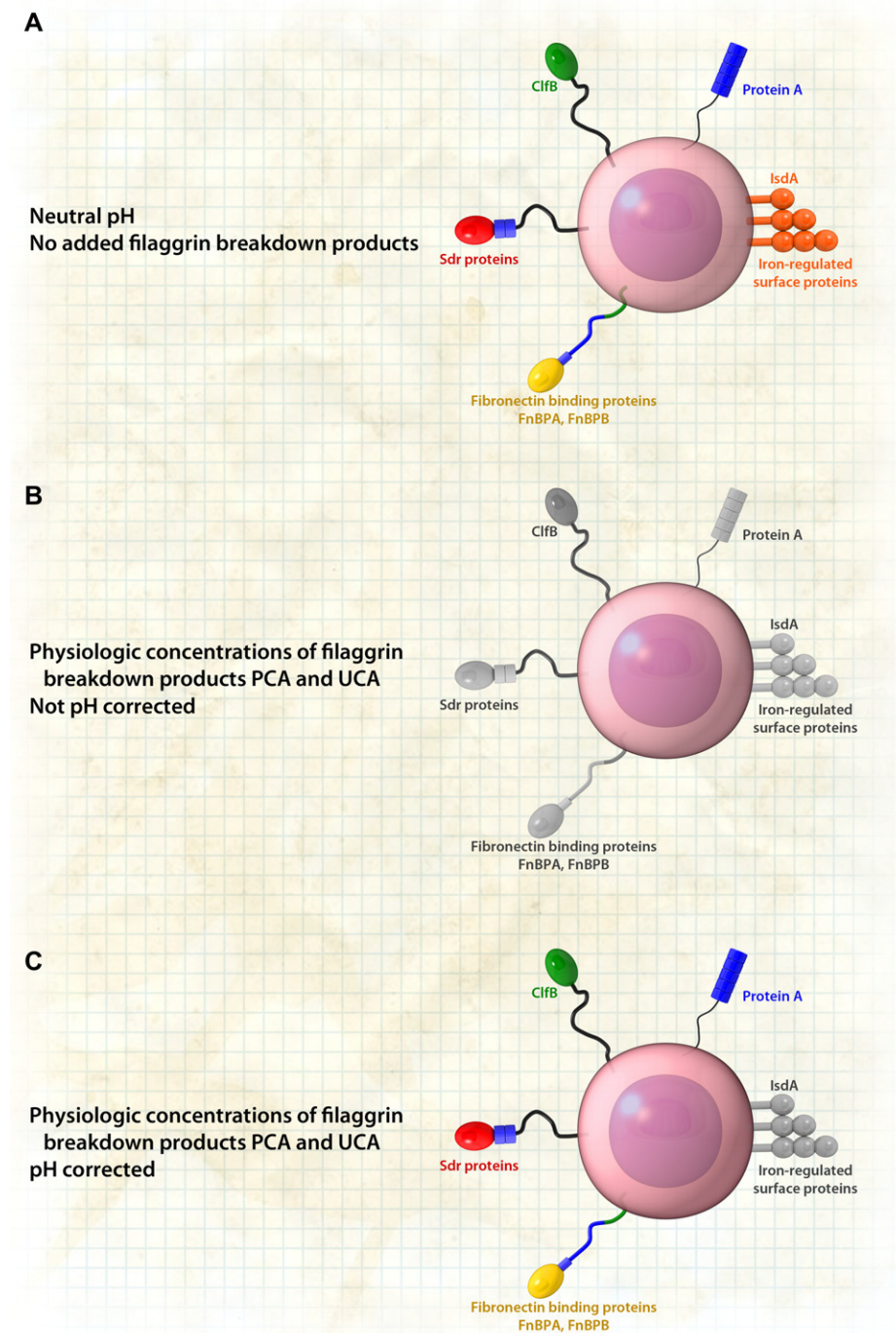


FIG 4. Filaggrin deficiency and susceptibility to *S aureus*. **A**, *S aureus* has a variety of bacterial surface-associated proteins that can bind to host adhesive molecules and promote colonization in the dysfunctional skin barrier of patients with AD. These surface-associated proteins also contribute to inflammation. **B**, Acidification of growth media by using a physiologic concentration of the filaggrin breakdown products UCA and PCA found in healthy skin in subjects with wild-type *FLG* resulted in reduced expression of secreted and cell wall-associated proteins, including proteins involved in colonization (clumping factor B and fibronectin-binding protein A) and immune evasion (protein A). **C**, Correction of pH after the addition of physiologic concentrations of filaggrin breakdown products resulted in restoration of all the surface protein expressions, except for IsdA, the expression of which was not restored. IsdA promotes adhesion to squamous cells and enhances survival on human skin. The expression of IsdA appears to be directly affected by the presence of UCA and PCA independently of pH. These *in vitro* studies suggest pathomechanisms, other than pH, through which reduced filaggrin expression can result in enhanced susceptibility to *S aureus* colonization.

stimulated a systemic $T_H17/IL-17$ immune environment and enhanced epicutaneous OVA-induced systemic T_H2 immune responses.¹²¹ These changes lead to an eosinophil-rich and neutrophil-predominant lung inflammation and airway hyperresponsiveness. This effect was significantly diminished when the *IL17A* gene was knocked out.¹²¹ These data suggest that SEB plays an important role in OVA-induced lung inflammation and airway hyperresponsiveness through an IL-17A-dependent pathway.¹²¹ Therefore superantigen SEB secreting *S aureus* could be important for the development of asthma in patients with AD, whose skin is often colonized with bacteria.

Recent work has demonstrated that filaggrin expression, as a result of keratinocyte differentiation, significantly inhibits *S aureus* α -toxin-mediated pathogenicity. Furthermore, α -toxin was particularly lethal to filaggrin-deficient epidermal cells in *fl/fl* mice. The protective effect of filaggrin against α -toxin was mediated through secretion of sphingomyelinase, an enzyme that reduces the number of α -toxin binding sites on the cell surface. The impaired host defense against *S aureus* α -toxin, resulting in enhanced cytotoxicity of α -toxin, potentially further exacerbates the compromised barrier in AD.^{FLG}¹²² These studies suggest that filaggrin-deficient SC might be particularly susceptible to *S aureus*. Whether targeting bacterial colonization early in the disease course of AD could halt the development of asthma in patients with AD remains to be investigated.

CONCLUSION

With the discovery of the role of *FLG* mutations and CNV, inherited SC epithelial barrier integrity is now recognized as a critical factor in the development of AD and subsequent allergic sensitization. This has directed the development of skin barrier-focused therapies. However, the pathomechanisms of AD are complex and include interplay between these and other epidermal structural abnormalities, immunologic dysregulation, and the microbiome. The relative roles of these major players in disease initiation, persistence and flare, and the development of atopic comorbidities are key areas for further investigation in our field. Genetic and environmental influences on filaggrin expression, as well as the dynamic, bidirectional cross-talk between the skin barrier and immune system are clearly complex and will require further clinical, genetic, and immunologic work to better understand these interactions and their relative influences on the many different clinical aspects of AD that affect our patients. New insights into the complex pathophysiology of this common and complex disease should allow more targeted treatments and a more individualized approach to treatment, as well as a preventative approach in at-risk subjects.

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