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### Review Series

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# Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases

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**Classic atopic dermatitis is complicated by asthma, allergic rhinitis, and food allergies, cumulatively referred to as atopic diseases. Recent discoveries of mutations in the filaggrin gene as predisposing factors for atopic diseases have refocused investigators' attention on epidermal barrier dysfunction as a causative mechanism. The skin's barrier function has three elements: the stratum corneum (air-liquid barrier), tight junctions (liquid-liquid barrier), and the Langerhans cell network (immunological barrier). Clarification of the molecular events underpinning epidermal barrier function and dysfunction should lead to a better understanding of the pathophysiological mechanisms of atopic diseases.**

## Introduction

Atopic dermatitis (AD) is a chronic relapsing eczematous skin disorder that is frequently associated with elevated serum IgE levels and a family history of AD, allergic rhinitis, and/or asthma. Clinical manifestations of classic AD are dry skin and relapsing eczema, which usually start during early infancy or childhood and become complicated by food allergies, asthma, and/or allergic rhinitis during the first several years of life, in a process called "atopic march" (1). AD is highly prevalent in industrialized countries, where it affects approximately 15%–30% of children and 2%–10% of adults (2). The various observations of the disease indicate that AD has a complex etiology with genetic, immunological, and environmental aspects.

Living organisms rely critically on surface barriers to isolate themselves from the external environment and to maintain homeostasis. While unicellular organisms are enclosed by cell membranes and cell walls, epithelial barrier structures, in several forms, cover the surfaces of multicellular organisms (3). In mammals, the airway and gastrointestinal tract are lined by simple epithelia covered with mucus. In contrast, the outer surface of the body is covered by a stratified epithelial cellular sheet called the epidermis, the outermost layer of which is cornified. Recent findings have shown that disruption of epithelial barrier systems are involved in the pathogenesis of immune disorders such as inflammatory bowel disease, asthma, and AD (4–12). In this review, we describe the barrier system of the epidermis, which is far more sophisticated than previously thought (13, 14), and attempt to discuss its function with special focus on antigen penetration through these barriers and antigen capture by dendritic cells in the context of AD.

## The three musketeers of the epidermal barrier

*Tight junctions as a liquid-liquid interface barrier.* For cellular sheets to function as proper barriers, paracellular diffusion of fluid must be prevented. In simple, single-layer cellular sheets in vertebrates, tight junctions (TJs) are responsible for intercellular sealing and the compartmentalization of extracellular environments (ref. 3 and Figure 1A). TJs are not just physical barriers; they exhibit ion and size selectivity and their barrier function varies significantly in

tightness, depending on cell type and physiological requirements, enabling dynamic regulation of substances that traffic between compartments. As a result, two adjacent compartments divided by TJ barriers can maintain different ionic strengths and solute concentrations (15–17).

Within the body of vertebrates, the TJ barrier acts as a liquid-liquid interface barrier to demarcate different fluid compartments. Examples of the compartmentalized fluids separated by TJ barriers are bile (in the lumen of bile ducts), urine (in the renal tubules), and cerebrospinal fluid (within the blood-brain barrier). TJs exist also in the body surface, both in the simple epithelia of body cavities such as intestine and trachea and in the stratified epithelia of skin. In the non-keratinized epidermis of fish and amphibian tadpoles, TJs separate the body from the external aquatic environment (18, 19). In terrestrial vertebrates, in which stratum corneum (SC) evolved presumably as an air-liquid interface barrier to avoid desiccation as animals adapted to living in contact with air, TJ barriers exist covertly under the SC barriers (18, 20–24). They compartmentalize the epidermal extracellular fluid environment into two parts in mammals (described below) and possibly also in reptiles and birds (Figure 1B).

Mammalian epidermis consists of a single layer of proliferating cells, the stratum basale, and several superficial layers of stratum spinosum and stratum granulosum (SG), which ultimately form the SC. As thin as it is, mouse ear epidermis has the minimal components of epidermal differentiation, with pre-SC cell flattening always occurring in three layers in the SG (25–27). The three SG layers are designated SG1, SG2, and SG3, counting from the surface inward. Our imaging studies of whole-mounted epidermal sheets have demonstrated that the intercellular spaces between SG2 cells are sealed by TJs (ref. 13 and Supplemental Video 1; supplemental material available online with this article; doi:10.1172/JCI57416DS1), as previously proposed based on careful observations via immunofluorescence and electron microscopy (22). Thus, SG1 cells that are about to cornify exist within a distinct fluid compartment outside of the TJ barrier, and cells below the SG2 layer are inside the barrier (Figure 1B).

*The SC as an air-liquid interface barrier.* As spinous layer cells differentiate into SG cells, they become flattened, with diameters up to 30 micrometers (27), and start to form lamellar and keratohyalin granules (Figure 1C). When SG3 cells differentiate into SG2 cells, they form TJs and acquire an apical-basal polarization of

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the cell membrane (13, 22). From the apical membrane of SG2 cells, which correlate with the secretory granulocytes observed by electron microscopy (28), the contents of lamellar granules (e.g., lipids, extracellular structural proteins, hydrolytic enzymes, and various antibacterial peptides such as cathelicidin and  $\beta$ -defensin 2; refs. 29–31) begin to be secreted into, and are kept within, the extra-TJ compartment, where they form the intercellular structures of the SC (32). After SG2 cells differentiate into SG1 cells, they appear to lose their TJs and initiate the final cornification steps of terminal differentiation, which occur outside the TJ barrier within a distinct fluid compartment that is probably required for terminal differentiation. Based on the results of electron microscopic studies and immunofluorescence studies, the observed “transient cells” (33) are likely equivalent to SG1 cells (13, 22) undergoing cornification.

During cornification, SG1 cells are enucleated, lose intracellular organelles and cell membranes, and are encapsulated in a heavily cross-linked proteinous structure called the “cornified envelope” (34). Within the extra-TJ compartment, the adhesion complexes between SG1 cells, desmosomes, are modified to corneodesmosomes through the incorporation of corneodesmosin (29, 35). Paracellular spaces outside of the TJ start to be filled with a mixture of relatively nonpolar lipids enriched in ceramides, cholesterol, free fatty acids, and cholesterol esters, which are secreted from the apical ends of SG2 cells. These lipids form intercellular lipid lamellae, or the “mortar” of the SC. SG1 cells transform into the lowermost corneocytes, which are the “bricks” surrounded by the mortar. Multiple layers of this structure form the SC, outermost bricks of which are desquamated in a well-controlled process (described below).

*Langerhans cells as an immune barrier.* DCs are leukocyte subsets that specialize in antigen presentation and are distributed throughout the body to protect the body from microbial or antigenic insult. Several subsets of DCs reside in skin, and the Langerhans cells (LCs) are unique DCs of the epidermis, distinct from several subsets of dermal DCs (36, 37). Although LCs were long believed to be the initiators of immune responses in skin, several studies utilizing transgenic mice that allow selective targeting of DCs failed to demonstrate their critical contribution in hapten-induced contact hypersensitivity models (36, 37). However, more recent studies have demonstrated that LCs are indeed capable of inducing antigen-specific Th2 responses in vivo (38, 39).

Penetration of the TJ barrier by DC dendrites and subsequent uptake of extra-TJ antigens have been reported in the lamina propria of the intestine and bronchial epithelium and may also occur in nasal and adenoid epithelia (40–46). The epidermis, because of its stratification and two sets of barriers, is quite different from simple epithelia with regard to antigen penetration and the capture of antigens by DCs. Under steady-state conditions, LCs are on standby with their dendrites aimed outward, positioned close to, but never crossing, the TJ barrier (Figure 1B). SC barrier perturbation (e.g., tape-stripping or acetone treatment) has been known to stimulate cytokine production in the epidermis and/or to activate LCs (47–50). Once activated, LCs extend their dendrites through TJ barriers and take up antigens from the extra-TJ environment (Figure 2, Supplemental Video 2, and ref. 13). During this process, the tightness of the epidermal TJs is maintained by the de novo formation of TJs between keratinocytes and LCs, suggesting that LCs are able to take up foreign antigens from outside TJ barriers without penetrating these barriers (13). In contrast, dermal DCs

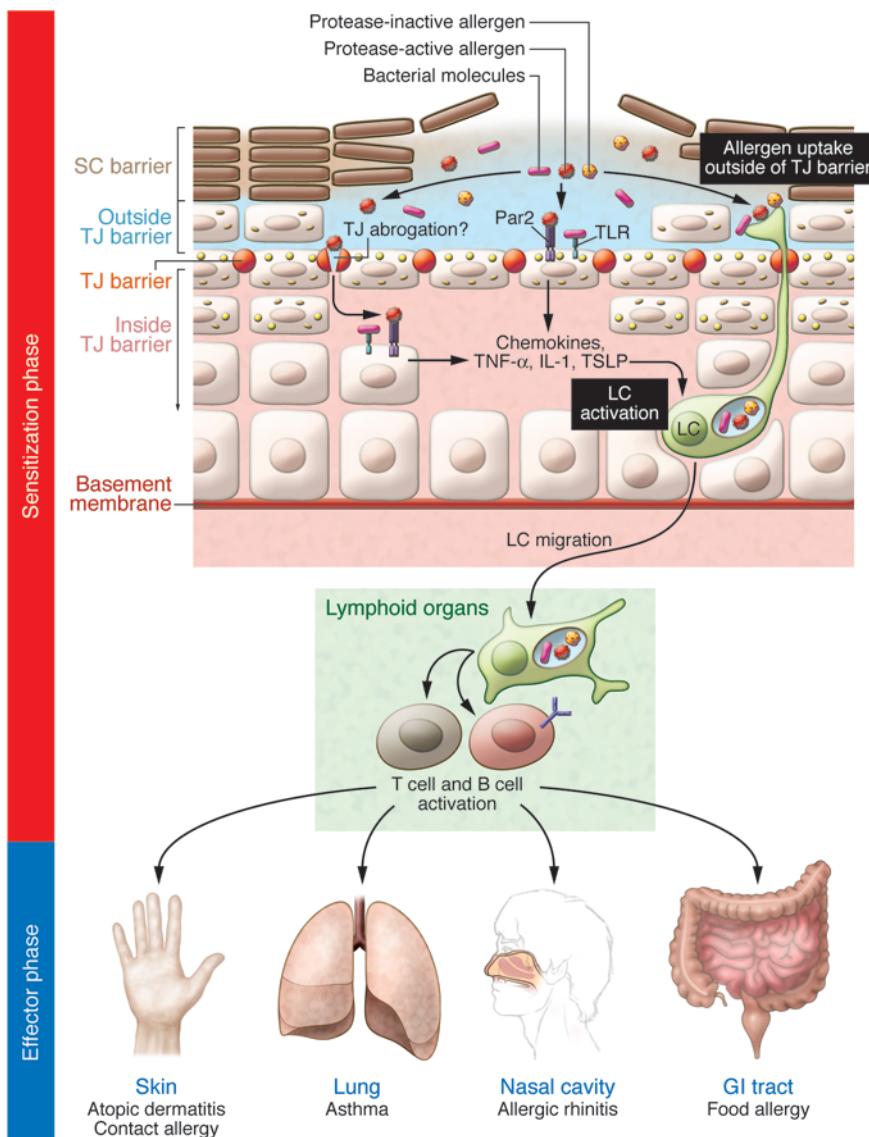
presumably take up antigens that have penetrated both epidermal barriers. Ouchi et al. recently showed that antigen uptake through TJs by LCs leads to a protective Th2 (IgG1) humoral response in a mouse model of staphylococcal scalded skin syndrome (51). At present, it appears that LCs only induce a Th2 response in the presence of an intact TJ barrier. It would be of interest to explore the deviation of immune responses that is induced upon the disruption of TJ barriers, which might allow dermal DCs to have access to antigens, thus leading to immune responses not restricted to Th2. It will be important to assess such immune responses utilizing protein antigens that do not easily pass through TJs, instead of haptens that readily penetrate the epidermis, regardless of the barriers, to the dermis.

*Dynamic cooperation and interaction of the three musketeers.* As described above, the SC, TJs, and LCs together constitute a skin surface surveillance and barrier system. The SC directly faces the external environment, protecting the inner viable layers from desiccation. The SC is not a sterile surface but controls skin flora of microorganisms via antimicrobial peptides and low pH (52). The SG1 layer exists outside of the TJ barrier and its extracellular space is rich in various proteases, antimicrobial peptides, and, under certain circumstances, bacteria and bacteria-derived metabolites/antigens. TJs in the SG2 layer compartmentalize this layer from the viable layer, preventing the potentially pro-inflammatory molecules above it from perturbing immune signals (see below). LCs, positioned covertly under the TJs, are polarized to send their dendrites outside of the TJ barrier when activated by danger signals such as TSLP, TNF- $\alpha$ , and IL-1 $\beta$ , presumably secreted from keratinocytes that somehow detect SC damages (Figure 2 and refs. 47, 53–56). The SG1 layer allows LCs to sample for foreign materials that have violated the SC barrier (13). Thus these three musketeers work all for one, and one for all, to provide a highly organized skin barrier.

### Major players in SC barrier dysfunction

*Filaggrin mutations as predisposing factors for atopic diseases.* Filaggrin is expressed in SG layers as a >400-kDa precursor protein, profilaggrin. Profilaggrin is the major component of the keratohyalin granules in SG cells. It is composed of highly homologous tandem repeats of the filaggrin monomer (10–12 in humans and 16 in C57BL/6 mice), flanked by two imperfect repeats and N- and C-terminal domains. Profilaggrin is proteolyzed into filaggrin monomers and N- and C-terminal peptides at the boundary of the SC and SG, possibly in SG1 cells. The functions of the N- and C-terminal peptides are as yet unknown, but nuclear translocation of the former, which contains an S-100 Ca<sup>2+</sup>-binding domain, has been reported (57). Filaggrin monomers display keratin-binding activity in vitro and have been proposed to promote the compaction of corneocytes by contributing to keratin pattern formation in the lower SC (58). Filaggrin monomers are further degraded into natural moisturizing factors (NMFs) that are thought to maintain hydration of the upper SC and to reduce the pH of the skin surfaces (59–61).

Mutations in the filaggrin (*FLG*) gene were identified initially as the cause of ichthyosis vulgaris and subsequently as a major predisposing factor for AD (Table 1 and refs. 62, 63). More recent studies have confirmed the association and identified additional mutations in the *FLG* gene that predispose to AD in European and Asian populations (64–67). *FLG* mutations have also been reported to be associated with atopic asthma, allergic rhinitis, nickel allergy,

**Figure 2**

A model of barrier disruption and consequences of percutaneous sensitization. SC barrier damages induce danger signals in the epidermis. After SC barrier abrogation, protease-active allergens and uncontrolled intrinsic proteases, as well as bacterial molecules such as lipoteichoic acid of gram-positive bacteria, might agonize Par2 and TLRs on keratinocytes, respectively (7, 55, 112, 123). Keratinocytes then produce TNF- $\alpha$ , IL-1, and thymic stromal lymphopoietin (TSLP) (47, 55), in response to which LCs become activated (54, 56, 124). Alternatively, protease-active allergens might directly obscure the TJ barrier and then penetrate the epidermis (7), where they directly or indirectly activate LCs. Upon SC perturbation, dendrites of activated LCs penetrate the TJs to take up protease-active or -inactive antigens from the extra-TJ environment (13). Such percutaneous sensitization and chronic allergen challenge via different routes, such as the lungs, nasal cavities, and intestinal tract, are speculated to manifest as the atopic march.

and food allergy (68–73), suggesting that *FLG* mutation-associated SC barrier defects lead to increased numbers of episodes of percutaneous allergen exposure (Figure 2). Interestingly, *FLG* mutations are not associated with asthma without eczema (70, 71), and most of the identified asthma-associated genes are not associated with AD (74, 75), suggesting that atopic asthma is a distinguishable sub-entity. Because filaggrin is not expressed in the upper airways (76), systemic sensitization is likely attributable to percutaneous antigen exposure through filaggrin-deficient skin. In good agreement with this hypothesis, percutaneous sensitization induces allergic responses to allergens in mice challenged via the upper airway (77, 78). Further investigation is required to clarify the impact of filaggrin deficiency on the barrier function of SC and the molecular mechanisms of percutaneous sensitization in the pathophysiology of AD and atopic diseases.

**Mouse models of filaggrin deficiency.** Flaky tail (*ft*) mice were created in 1958 on the background of an existing recessive hair phenotype, matted (*ma*), and also exhibited dry, flaky skin with annular tail and paw constrictions during the neonatal period (79). Since

then, they have been maintained in a mixed strain (*ft/ma*) (80). The *ft* mutation was found to be a 1-bp deletion in filaggrin repeat 6, which leads to premature termination, analogous to common human *FLG* mutations (81). *ft/ma* mice show paracellular barrier permeability in SC (82), increased trans-epidermal water loss (TEWL), and enhanced percutaneous allergen priming (83). They also spontaneously develop dermatitis under steady-state conditions (83) and have been used as a model of filaggrin deficiency and AD. However, *ma* locus-eliminated homozygous *ft* mice (identified by the absence of matted hair) only show mild diffuse hypertrophy of the SC and sporadic superficial dermal cellular infiltrates under steady-state conditions (81). Furthermore, while *ft/ma* mice show elevated TEWL (83), *ft* mice show no statistically significant difference in baseline TEWL (81). Thus, when *ft/ma* mice are used in studies of AD in the context of filaggrin deficiency, the results need to be interpreted carefully. It would be of interest to identify the gene responsible for the *ma* phenotype, and the gene's function, to understand the pathophysiological mechanisms behind the spontaneous development of dermatitis in *ft/ma* mice.



**Table 1**  
Skin barrier–related genes associated with AD/AD-like dermatitis

Gene symbol	Gene name	Functions	Representative refs. <sup>A</sup>
Filaggrin system			
<i>FLG</i>	Filaggrin	Major constituent of keratohyalin granules; bundling keratin filament to form keratin pattern; degradation products are reported to have skin-moisturizing activity	62, 69
Desquamation			
<i>SPINK5</i>	Serine peptidase inhibitor, Kazal type 5	pH-dependent inhibition of KLK5 and KLK7	86
<i>KLK7<sup>B</sup></i>	Kallikrein-related peptidase 7	Digestion of corneodesmosin	96
<i>CDSN</i>	Corneodesmosin	Structural protein of corneodesmosomes	97 <sup>C</sup>
Others			
<i>CSTA</i>	Cystatin A	Cysteine protease inhibitor of house dust mite protease	125
<i>CLDN1<sup>D</sup></i>	Claudin 1	Integral transmembrane protein of TJs	109

<sup>A</sup>For more detailed data on gene variants, see ref. 75. <sup>B</sup>The association with AD is still controversial (126). <sup>C</sup>Only reported as responsible for peeling skin syndrome type B. <sup>D</sup>No atopic disease has been reported to complicate in patients with NISCH, who totally lack claudin 1 protein (107, 108).

*Hereditary SC barrier deficiency with complicating atopic disease.* Desquamation is a well-orchestrated process controlled by a network of proteases, which themselves are regulated by protease inhibitors and skin pH. Among these, kallikrein-related (KLK-related) peptidases and their pH-dependent inhibition by lymphoepithelial Kazal type–related inhibitor (LEKTI; encoded by serine peptidase inhibitor, Kazal type 5 [*SPINK5*] gene) are the best characterized (Figure 1B and refs. 84, 85). Under normal conditions, the neutral pH in the lower SC enables LEKTI to inactivate KLK5 and KLK7, while the acidic conditions in the uppermost part of the SC inhibit LEKTI, thereby allowing KLK5 and KLK7 to function and thus promoting skin peeling. Genetic studies have demonstrated linkage between polymorphisms in *SPINK5* and AD, asthma, and elevated levels of serum IgE (86–88). Loss of function of *SPINK5* causes Netherton syndrome, a severe autosomal recessive ichthyosis characterized by chronic dermatitis, asthma, and allergic rhinitis (89). Ultrastructural analyses show a marked increase in corneodesmosome cleavage and SC detachment at the border between transient cells and SG cells in the skin of patients with Netherton syndrome (90–92). Studies of *SPINK5*-knockout mice confirmed that LEKTI deficiency results in elevated KLK5 activity in the layers of the SC and subsequent breakdown of corneodesmosin, followed by SC barrier loss (93–95). Polymorphisms in *SPINK5* and *KLK7* associated with AD may produce a similar, but milder, SC barrier defect (Table 1 and refs. 86, 96). Genetic loss of corneodesmosin causes peeling skin syndrome type B, in which the whole SC is easily detached from the underlying living layers, leading to chronic dermatitis, asthma, allergic rhinitis, elevated levels of serum IgE, and food allergy (Table 1 and ref. 97), suggesting that SC barrier dysfunction precedes the onset of atopic diseases.

Loss of the SC barrier, as occurs in Netherton syndrome and peeling skin syndrome type B, induces dermatitis, asthma, and allergic rhinitis, suggesting that perturbation of the desquamation processes could induce SC barrier defects and predispose to AD or similar conditions. Overexpression of elastase 2 and over degradation of profilaggrin were recently reported in the skin of patients with Netherton syndrome (98), and it is necessary to evaluate whether the filaggrin system malfunction plays a direct role on the barrier dysfunction in these two diseases. Interestingly, under LEKTI-deficient conditions (as in Netherton syndrome),

spontaneous protease-activated receptor 2 (Par2) activation and subsequent production of thymic stromal lymphopoietin, IL-8, and TNF- $\alpha$  in keratinocytes have been reported to result from uncontrolled activation of KLK5, indicating that aberrant activation of barrier-associated proteases can modulate immunity in ways that exacerbate atopic manifestations (55).

*Search for additional candidate genes in atopic diseases.* As mentioned above, filaggrin mutations are, thus far, the major predisposing factor for classic atopy. However, such mutations are not found in a significant number of patients, indicating that other genetic factors that influence skin barrier function may also be important. Candidate molecules include enzymes that function in the degradation of profilaggrin (61). Retroviral-like aspartic protease (SASPase) is a skin-specific protease that directly cleaves a linker peptide in profilaggrin. Hairless mice deficient in SASPase exhibit dry skin and aberrantly processed profilaggrin accumulation in the lower SC (99). Caspase-14 is a skin-specific enzyme that may be involved in the early degradation of filaggrin monomers to NMFs, and caspase-14-knockout mice display a dry skin phenotype (100, 101). Bleomycin hydrolase has been reported to be involved in a later stage of the degradation processes (102, 103). Bleomycin hydrolase-knockout mice display generalized scaling and mild ichthyosis in their neonatal period, while adults appear normal but develop low-humidity-induced dermatitis in their tails (104). Further epidemiological studies are warranted to determine whether mutations in these proteases are predisposing factors for human AD.

TJ barriers may also be involved in proper SC formation. Claudin 1 is one of the major integral transmembrane components of epidermal TJs, and in claudin 1-knockout mice, epidermal TJs lose tightness and the SC displays compact hyperkeratosis, suggesting that loss of tightness of the TJ barrier may also affect terminal differentiation in the epidermis (22). Ectopic overexpression of periderm-specific claudin, claudin 6, in the SG induces a phenotype that is identical to claudin 1-knockout mice (105, 106), suggesting that differential expression or the balance of claudin molecules might regulate the tightness of TJ barriers. Patients with neonatal ichthyosis and sclerosing cholangitis (NISCH) have homozygous nonsense mutations in the claudin 1 gene and display ichthyosis, further suggesting the



involvement of the TJ barrier in proper SC formation (107, 108). Interestingly, a polymorphism in the claudin 1 gene was recently reported as a possible candidate mutation in AD (Table 1 and ref. 109). Further large-scale epidemiological studies and careful investigation of the crosstalk between the TJ and SC barriers are necessary to assess the contribution of TJs to the pathogenesis of AD.

### Percutaneous sensitization through insufficient SC barrier

*LC activation induced by SC barrier perturbation.* In contrast to the above-described congenital diseases that affect the SC, the entire epidermis is lost in junctional or dystrophic epidermolysis bullosa (EB) (110). Interestingly, patients with EB do not usually develop atopic diseases, and genes responsible for EB, such as those that encode laminin 332, and the type VII and XVII collagens, were not identified in genome-wide linkage studies of AD or asthma. Thus, living layer structures of the epidermis and cellular immune components, such as LCs, may be required for the development of atopic diseases. In this context, it is important to understand how foreign antigens or allergens are taken up within the epidermis and presented to the immune system (12, 51).

To induce epicutaneous sensitization to protein antigens in mice, the tough SC barrier needs to be mechanically impaired by tape-stripping, acetone treatment, or patch dressing (78). Interestingly, tape-stripping mouse ear skin activates LCs and induces penetration of the TJ barrier by LC dendrites, suggesting that causing physical stress to the SC induces danger signals in epidermis that directly or indirectly activate LCs (Figure 2 and refs. 13, 47, 50, 53–56). Thus, perturbation of the SC barrier not only allows allergen penetration throughout this barrier, but also triggers LC activation and facilitates subsequent uptake of antigens by LCs across the epidermal TJ barrier. After antigen acquisition, LCs presumably migrate to draining lymph nodes and activate antigen-specific T cells. Although danger signals similar to those observed in simple epithelium models, such as the lungs, may also occur in skin, as shown in Figure 2 (7, 53, 111–114), it is not yet clear how LCs sense danger signals caused by SC damage, and the specific signals that are delivered across the TJ barrier remain to be investigated.

*The chicken or the egg in atopic diseases.* Skin barrier defects and immune disorders present a chicken and egg dilemma in AD. Allergens that have penetrated defective skin barriers induce inflammation, while inflammation itself can alter skin barrier integrity. Th2 and Th17 cytokines have been reported to down-regulate filaggrin expression or proper processing of profilaggrin (115–119). Not only genetic skin barrier defects, but also genetic immune disorders such as Wiskott-Aldrich syndrome, show atopic manifestations (120), indicating that AD emerges in the context of both epidermal barrier- and immunity-asso-

ciated genetic dysfunction. Indeed, many immunity-associated genes, e.g., Th2 and Th17 cytokines, innate immune receptors, and various chemokines, have been identified through whole-genome linkage studies of susceptibility genes for atopic diseases (75), some of which have been reported to show a multiplicative effect with *FLG* mutations on AD (121). It seems reasonable to suggest that immunity-associated genetic factors, as well as environmental factors, may act additively (or synergistically) to produce SC barrier defects and to promote percutaneous sensitization during the onset of AD.

### Conquering the epidermal barrier is a double victory

Epidermal barriers face harsh challenges in modern life. Features of current lifestyles, such as frequent bathing and regular use of soap, and living within concrete jungles with air conditioning that dehumidifies the air, may accelerate SC barrier impairment (11). Constant heating, decreased ventilation, and floors covered with carpets are likely to increase house dust mite numbers and allergen levels in living environments. These environments, which expose the skin to both mechanical and allergic cues, may induce not only SC barrier damage but also danger signals that activate LCs, and could be a part of the reason why the prevalence of atopic diseases is increasing in industrialized countries (2). *FLG* mutations are associated with not only AD, but also atopic asthma, allergic rhinitis, and food allergy, emphasizing the importance of percutaneous sensitization as an initial trigger of atopic cascades, which lead to the atopic march (Figure 2). A simple and straightforward approach to preventing or controlling atopic cascades is to maintain intact epidermal barriers after birth or after improvement of acute inflammation (122). Clarification of the exact molecular functions of the epidermal barrier, with the SC as an air-liquid interface barrier, TJs as a liquid-liquid interface barrier, and LCs as an immunological barrier, will lead to a more sophisticated understanding as well as practical solutions for conquering atopic diseases.

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