Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases

Akiharu Kubo,1,2 Keisuke Nagao,1 and Masayuki Amagai1

1Department of Dermatology and 2Center for Integrated Medical Research, Keio University School of Medicine, Tokyo, Japan.

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 complexity of skin. In the non-keratinized epidermis of fish and amphibian tadpoles, TJs separate the body from the external aquatic environment. 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. 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).

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...
Figure 1
Schematic representation of the barriers in simple epithelia and stratified epidermis. (A) In simple epithelia, TJs seal the apical end of the lateral cell membrane. The extracellular fluid is compartmentalized into two parts by TJs. (B) In the mammalian epidermis, the SC serves as an air-liquid interface barrier and protects the living layers from desiccation. TJs also seal the paracellular spaces between SG2 cells. TJs act as a liquid-liquid interface barrier in both simple and cornified stratified epithelia. LCs position their dendrites upward, ready to survey antigens upon sensing perturbation. (C) Terminal differentiation in relation to TJs. When SG3 cells differentiate into SG2 cells, they form TJs (i) and begin to secrete lamellar granules from their apical membranes (ii). SG1 cells appear to lose their TJs (iii) and then undergo final cornification (iv). Mature corneocytes are encapsulated in the cornified envelope (dark brown; v), and their intercellular spaces are filled with lipid lamellae (brown). Corneodesmosomes (green squares; vi) mediate intercorneocyte adhesion. KLKs secreted into the extracellular space are strictly limited to the extra-TJ environment. As the pH becomes acidic in the upper layers of the SC, KLKs are released from LEKTI and proteolyze corneodesmosomes, initiating desquamation (vii). Profilaggrin is a component of keratohyalin granules in the SG, is degraded into filaggrin monomers, possibly in SG1 cells, and is further degraded into NMFs in the upper SC (viii).
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, hydrolitic enzymes, and various antibacterial peptides such as cathelicidin and β-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 TJ s 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 cornocytes, 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 subsets that specialize in antigen presentation and are distributed

**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 comprised 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 Ca2+–binding domain, has been reported (57). Filaggrin monomers display keratin-binding activity in vitro and have been proposed to promote the compaction of cornocytes 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,
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 filagrin-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.

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-α, 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.
review series

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

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Functions</th>
<th>Representative refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLG</td>
<td>Filaggrin</td>
<td>Major constituent of keratohyalin granules; bundling keratin filament to form keratin pattern; degradation products are reported to have skin-moisturizing activity</td>
<td>62, 69</td>
</tr>
<tr>
<td>Desquamation</td>
<td>Serine peptidase inhibitor, Kazal type 5</td>
<td>pH-dependent inhibition of KLKS and KLK7</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>KLK7⊙</td>
<td>Digestion of corneodesmosin</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>CDSN</td>
<td>Structural protein of corneodesmosomes</td>
<td>97c</td>
</tr>
<tr>
<td>Others</td>
<td>CSTA</td>
<td>Cysteine protease inhibitor of house dust mite protease</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>CLDN1○</td>
<td>Integral transmembrane protein of TJs</td>
<td>109</td>
</tr>
</tbody>
</table>

*For more detailed data on gene variants, see ref. 75. ⊙The association with AD is still controversial (126). ○Only reported as responsible for peeling skin syndrome type B. □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 KLKS activity in the layers of the SC and subsequent breakdown of corneodesmosine, 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 corneodesmosine 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-α 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 as a possible candidate mutation in AD (Table 1). Phenotypic expression of Langerhans cells of hyperplastic skin is associated with 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.

**Acknowledgments**

This work was supported by Grants-in-Aid for Scientific Research and the “Promotion of Environmental Improvement for Independence of Young Researchers” program funding from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by Health Labour Sciences Research Grants for Research on Allergic Diseases and Immunology from the Ministry of Health, Labour, and Welfare of Japan.

Address correspondence to: Masayuki Amagai, Department of Dermatology, Keio University School of Medicine, Shinanomachi 35, Shinjuku, Tokyo 160-8582, Japan. Phone: 81.3.53633822; Fax: 81.3.33516880; E-mail: amagai@7.keio.jp.


