



Abnormal epidermal barrier in the pathogenesis of atopic dermatitis

Ronni Wolf, MD^{a,*}, Danny Wolf, MD^b

^a*Dermatology Unit, Kaplan Medical Center, Rehovot 76100, Israel*

(affiliated to the Hebrew University—Hadassah Medical School, Jerusalem, Israel)

^b*Sherutei Briut Clalit, Health Care, Hasharon Region, Natanya, Israel*

Abstract Despite the acknowledged contributions of a defective epidermal permeability barrier, dryness of the skin, and the propensity to develop secondary infections to the etiology and pathophysiology of atopic dermatitis (AD), these epidermal changes have, until recently, been assumed to reflect downstream consequences that are secondary phenomena of the primary immunologic abnormality—the historical “inside-outside” view that AD is basically an intrinsic inflammatory disease.

In this review, we focused on the role of the epidermal barrier function in the pathophysiology of AD. Specifically, we presented data in support of a barrier-initiated pathogenesis of AD, ie, the “outside-inside” concept. First, we reviewed the evidence on the existence of inherited barrier abnormalities in AD. Reported studies on the possible association of mutations in the filaggrin gene (*FLG*) and data on human tissue kallikreins (KLKs) and AD have been addressed. We then dealt with the question of the causal link between impaired epidermal barrier and inflammation. Finally, the association between innate immune defense system and the increased avidity of *Staphylococcus aureus* for atopic skin was examined.

Despite very convincing evidence to support the barrier-initiated pathogenesis of AD, the view that AD reflects the downstream consequences of a primary immunologic abnormality cannot be dismissed out of hand. Almost every line of evidence in support of the role of the epidermal barrier as the “driver” of the disease activity can be challenged and at least partially contradicted by opposing evidence.

Until more data are available and until all the dust settles around this issue, we should take advantage of what we already know and use our knowledge for practical purposes. Deployment of specific strategies to restore the barrier function in AD means the use of moisturizers as first-line therapy.

© 2012 Elsevier Inc. All rights reserved.

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory disease associated with considerable psychosocial morbidity, impairment in quality of life, and a heavy economic burden, not only on patients and their families but on society as a whole.

Despite the acknowledged contributions of a defective epidermal permeability barrier, dryness of the skin, and the propensity to develop secondary infections to the etiology and pathophysiology of AD, these epidermal changes have,

until recently, been assumed to reflect downstream consequences that are secondary phenomena of the primary immunologic abnormality—the historical “inside-outside” view that AD is basically an intrinsic inflammatory disease. The rapid and impressive advances in immunology and immune-targeted therapies have now heightened our awareness to immune abnormalities, such as increased serum immunoglobulin (Ig)E levels and sensitizations to allergens, increased T-helper type 2 (T_H2) cytokine expression, and the active role of Langerhans cells and antigen-presenting cells, eosinophils, and many others, all leading to the consensus that immunology is the central and primary event of AD.

The question of which came first, the advent of immunologic-targeted therapies (eg, cyclosporine, calci-

* Corresponding author. Fax: +972-9-9560978.

E-mail address: wolf_r@netvision.net.il (R. Wolf).

neurin inhibitors, the “biological,” and others) or the detection of immunologic abnormalities through basic research, is shrouded in mystery. What is clear, however, is that both are involved in creating the so-called “inside-outside” theory, specifically, that AD is first and foremost an immunological disease and that immunological abnormalities are the primary, the initializing, and triggering events. For example, in the 2008 edition of Fitzpatrick’s *Dermatology in General Medicine*, AD is discussed under the section of “Inflammatory disorders based on T-cell reactivity and dysregulation,”¹ although a considerable part of the discussion of the etiology in this chapter is devoted to impairment of epidermal barrier function.

We have recently been feeling the winds of change in our views on disease pathogenesis. There has been a shift from immunological/biochemical mechanisms toward a theory that the epidermal abnormality is not merely a secondary phenomenon resulting from the infiltration of abnormal immune and inflammatory cells and their mediators, but rather a critical, if not the primary, exacerbant of this inflammatory skin disease.

The current review is intended to provide insights into the unique and pivotal role of the epidermal barrier function in the pathophysiology of AD and its practical implications for therapy.

Inherited barrier abnormalities in atopic dermatitis: filaggrin deficiency

The term “filaggrin” is derived from “filament-aggregating protein,” and it describes the protein’s function of binding to keratin intermediate filaments, causing their aggregation into macrofibrils in which the intermediate filaments are aligned in tightly packed parallel arrays. This process contributes to cellular compaction and permits extensive crosslinking of keratin intermediate filaments by transglutaminases so as to form a highly insoluble keratin matrix. This matrix acts as a protein scaffold for the attachment of cornified cell envelope proteins and lipids that together form the stratum corneum (SC).²

Filaggrin is initially synthesized as profilaggrin, a greater than 400 kDa, highly phosphorylated, histidine-rich polypeptide. Profilaggrin is the main constituent of the electron-dense keratohyaline granules that are found within the granular layer of the epidermis. During later stages of epidermal terminal differentiation, profilaggrin is dephosphorylated and proteolysed into multiple filaggrin monomers in a multistep process. The free filaggrin binds to keratin and acts as an aggregator of this protein, and thus has an important role in building the epidermal barrier.²

The cleaved N-terminal S100-like calcium-binding domain of profilaggrin enters the nucleus, where it is postulated as having an additional role in regulating the terminal keratinocyte differentiation. Subsequently, the filaggrin peptide within the SC itself is progressively degraded by

posttranslational modification enzymes into a pool of hydrophilic amino acids, including urocanic acid, pyrrolidine carboxylic acid, and alanine. This combined pool of amino acids, their metabolites, and various ions make up what is known as the natural moisturizing factor (NMF). NMF is highly hygroscopic and plays a central role in maintaining hydration of the SC. NMF might also play a critical role in the maintenance of the pH of the skin by regulating key biochemical events, including protease activity, barrier permeability, and cutaneous antimicrobial defense, functions that are fundamentally linked and coregulated. The importance of filaggrin-derived breakdown products and the profound effect on barrier function in their absence is underscored by the remarkably short half-life of filaggrin, specifically, only 6 hours before full proteolysis.³

Histidine comprises one of the most abundant amino acids released by filaggrin (FLG) proteolysis. It is converted to *trans*-urocanic acid (*trans*-UCA) that helps the pH gradient of the epidermis. Decreased generation of FLG products could result in an initial increase in the SC pH, sufficient to activate multiple serine protease in the SC, all of which exhibit neutral-to-alkaline pH optima. If such a pH-induced increase in serine protease activity is prolonged, it could precipitate the downstream structural and functional alterations described below.

Based on the assumption that the pathogenesis of AD is primary immunologic, genetic research has focused for many years on genes associated with immunologic abnormalities. The discovery of the association of mutations in the filaggrin gene (*FLG*) and ichthyosis vulgaris (IV), and the common association between IV and AD, has sparked off vigorous research on *FLG* mutations in AD as well, leading to the accumulation of a considerable amount of data within a relatively short period. The conclusions of all the studies cited in 2 recent meta-analyses^{4,5} can be stated as follows:

1. The effect of *FLG* on eczema risk is higher than that of any other confirmed candidate gene for atopic diseases and one of the largest ever shown in the genetics of complex diseases.
2. The strongest associations for *FLG* within eczema are in dermatologist-diagnosed cases and in moderate-to-severe cases.
3. *FLG* deficiency predisposes to the particular asthma phenotype occurring in the context of eczema in contrast to the form of asthma not linked with eczema. Because *FLG* is not expressed in bronchial mucosa, transcutaneous sensitization is one suggested mechanistic possibility for *FLG* to confer asthma risk.

So far so good; however, the concept that the highly significant association between abnormalities in the epidermal barrier, specifically in filaggrin, and that the risk of early-onset, severe, persistent AD also points toward a causal relationship between the 2 (the “outside-inside” hypothesis) is still a matter of ongoing debate.

There are several arguments against the existence of a link between abnormal filaggrin and AD risk. Low filaggrin levels might also be caused by modulation of epidermal protein levels by T_H2-type cytokines. Indeed, it had been shown that skin expression of *FLG* mutations can be downregulated by the T_H2 cytokines, interleukin (IL)-4 and IL-13.⁶

A significant number of patients with AD (reportedly 14% to 56% of European patients⁷) do not have any of the known *FLG* mutations and, conversely, approximately 40% of patients with *FLG*-null alleles never develop AD.⁸

Although several authors⁹ showed a strong correlation between clinical severity of the disease and barrier impairment in AD patients carrying *FLG* mutations, others¹⁰ failed to identify any effect of *FLG* mutations on skin conditions as assessed by clinical scoring and measurement of transepidermal water loss.

Finally, IV patients have the same single- or double-allele *FLG* mutations that lead to reduced filaggrin content, but they do not always have inflammation.

Inherited barrier abnormalities in atopic dermatitis: protease/antiprotease expression

Human tissue kallikreins (KLKs) are a family of 15 secreted serine proteases (SP) belonging to the chymotrypsinlike serine endopeptidase family. Multiple kallikreins are expressed in the skin epidermis and its associated appendages in active KLK and inactive pro-KLK forms.

Proper desquamation (shedding of corneocytes at the skin surface) is crucial for maintaining a normal structure and thickness of the skin. It should be in balance with the constant de novo production of cells at the basal layer. Epidermal kallikreins KLK5, KLK5, KLK7, KLK13, and KLK14 degrade corneodesmosomes, which maintain adhesion between neighboring keratinocytes, and thus play a key role in the desquamation process.¹¹

Skin barrier function depends on the formation of mature lamellar membranes in the SC subsequent to proper extracellular lipid processing of lipid precursors secreted by lamellar granules (LGs), or lamellar bodies. Lipid precursors are released into the stratum granulosum (SG)/SC interface for processing by various enzymes into ceramides and free fatty acids. These lipid-processing enzymes are regulated by KLKs via proteolytic degradation.

Elevated SP activity probably provokes barrier abnormality by a second, unrelated mechanism that, by signaling of the plasminogen activator type 2 receptor (PAR2), downregulates lamellar body (LB) secretion, entombing these organelles in nascent corneocytes. Failure of LB secretion accounts for the global decrease in SC lipids in AD, which correlates with a decrease in extracellular lamellar bilayers in AD. Thus, increased SP activity alone can induce abnormalities that parallel those in AD, providing a mechanistic basis for the global reduction in extracellular lipids and further decline in ceramide levels that occur in AD.^{12,13}

It has also been shown that inhibition of SP activity by topical SP inhibitors (SPIs) accelerates barrier recovery after acute abrogation owing to enhanced LB secretion, another way in which SP influence skin barrier formation and function.¹²

The most compelling case for the role of excess SP activity in the pathogenesis of AD comes from Netherton syndrome, an autosomal recessive disorder caused by loss-of-function mutations in *SPINK5*, the gene encoding the SP inhibitor, lymphoepithelial Kazal-type trypsin inhibitor (LEKTI).¹⁴ This syndrome is characterized by severe AD, mucosal atopy, and anaphylactic reactions to food antigens. Residual LEKTI expression in Netherton syndrome correlates inversely with excess SP activity within the outer epidermis, resulting in a severe permeability barrier defect and dramatic thinning of the SC owing to unrestricted, SP-dependent degradation of lipid-processing enzymes and corneodesmosome constituent proteins, respectively.¹⁵

Identification of mutations in the *SPINK5* gene as the cause of Netherton syndrome led to the screening of this gene for polymorphism in patients with atopic eczema. Although patients with Netherton syndrome carry mutations in the *SPINK5* gene on both alleles, AD patients, who share common features with Netherton syndrome, show coding polymorphisms in *SPINK5*, which are expected to affect the functionality of LEKTI domains.

Although the association between mutations in the *SPINK5* gene with AD is much weaker than in the case of *FLG* mutations, several groups have been able to confirm an association between those mutations with AD,¹⁶⁻²⁰ whereas others could not,^{10,21} but could instead demonstrate an association between *SPINK5* and raised IgE serum levels.¹⁰

The inconsistent results in the link between mutations in genes encoding SP and SP inhibitors are because the currently identified mutations in these genes do not determine subsets of AD patients and that there are other yet unidentified mutations. Furthermore, it has been suggested that the level of protease activity at the SC is an important indicator of milder forms of barrier disruption unrelated to AD, including sensitive skin. Consequently, the control populations used in previously cited studies might have had other conditions associated with high SP levels, thereby minimizing the differences between AD patient and control groups.

Finally, there are several other studies demonstrating an increase in SP²² and a decrease in LEKTI²³⁻²⁵ in patients with AD.

Basis for inflammation in atopic dermatitis

FLG deficiency is associated with decreased downstream production of substrates for proteolytic processing and further deimination into polycarboxylic acids, such as pyrrolidine carboxylic acid and trans-urocanic acid (UCA), the inevitable consequence of which is an increase in the SC's pH.^{26,27} The increased pH results in activation of multiple SP in the SC, which all exhibit neutral-to-alkaline pH optima.

One important downstream consequence of increased SP activity is the generation of IL-1 α and IL-1 β by proteolytic cleavage of the 31-kDa IL-1 precursor into the 17-kDa mature bioactive form.²⁸ Thus, the increase in SP activity (induced by the increase in pH and other mechanisms) could generate 17-kDa active forms of these cytokines, which are considered the first step in the cytokine cascade leading to inflammation in AD.²⁹

As convincing as this hypothesis seems at first glance, there are still some uncertainties that need to be addressed. First, the role of the filaggrin-histidine-urocanic acid pathway in SC acidification has not yet been established.^{30,31} Second, although IV shows the same defect in *FLG*, inflammation is not a hallmark of that disease.

Another cause for inflammation is sustained antigen ingress through a defective barrier, leading to a Th2-dominant infiltrate and activation of SP by mite antigen with further damage to the barrier.^{32,33}

Antimicrobial barrier dysfunction

Similar to permeability barrier dysfunction, the antimicrobial barrier is compromised in AD, leading to bacterial colonization, which can act as antigens and/or super-antigens that augment/trigger antibody production.^{29,34} The best characterized of all the infectious causes found to affect patients with AD is *Staphylococcus aureus*. Approximately 90% of patients with AD are colonized with *S aureus*, whereas only 5% to 30% are colonized in a control or nonatopic population. In addition to higher colonization rates, up to 50% to 60% of the *S aureus* found on patients with AD are toxin producing. Once present on the skin, *S aureus* can mediate multiple inflammatory cascades. For example, staphylococcal toxins can activate T cells in a superantigen-driven fashion and induce IgE-specific responses. In fact, the levels of these specific IgE levels have been shown to correspond with disease severity. Additionally, bacterial superantigens can induce a state of glucocorticoid resistance,^{29,34} and most studies have demonstrated that anti-staphylococcal treatments reduce disease severity, although a recent updated Cochrane review failed to find any evidence for a beneficial role of anti-staphylococcal treatments in people with AD.³⁵ In conclusion, it is clear that *S aureus* is able to induce both nonspecific and specific inflammation in subjects with AD, including that associated with an immediate hypersensitivity reaction. These effects might, however, help initiate and potentiate the disease and might even modify responsiveness to frequently prescribed therapeutic agents (eg, steroids).^{29,34}

It has long been suggested that AD patients may have a constitutional defect of the skin that increases the avidity of *S aureus* for atopic skin. It was recently suggested that this increase in *S aureus* could result from failure of the innate immune defense system of atopic skin to restrict the growth of the organisms. Naturally occurring antimicrobial peptides

(AMPs), a critical component of this innate immune system, have been shown to provide mammalian skin with resistance to bacterial infection.³⁶ AMPs are produced and secreted by the mammalian epithelium, and they constitute a primary antimicrobial defense mechanism. Among the naturally occurring AMPs secreted by the human epidermis, human β -defensin-2 (HBD-2) and IL-37, a cathelicidin that is packed in the LB, are major AMP with broad-spectrum antimicrobial activities.^{37,38}

The AMPs are normally produced by keratinocytes in response to inflammatory stimuli, such as psoriasis or injury. Ong et al³⁹ were the first to recognize that subjects with AD had reduced HBD-2 epidermal immunoreactivity compared with levels of subjects with psoriasis. Their finding was later confirmed by several other studies,²⁹ but expression of AMP at baseline was not different between the lesion-free skin of atopic individuals and the normal skin of nonatopic individuals.^{40,41}

These and other experiments brought the researchers to the conclusion that AMPs are deficient in the skin of AD patients, and that they play a role in the propensity of patients toward skin infection and colonization of *S aureus*, which can act as a superantigen and thus initiate the disease.

The reduced expression of AMP is probably not a result of a primary defect of the epidermis, but rather a result of the inhibitory effects of the T_H2 cytokines (IL-4 and IL-13)³⁹ and the immunomodulatory cytokine IL-10 on keratinocytes.⁴²

Because AMPs are normally produced by keratinocytes in response to inflammatory stimuli and are expressed only at low levels under basal conditions, the importance of epithelial integrity in the protection against pathogen assault cannot be overemphasized.²⁶ An intact SC deploys lipids with substantial antimicrobial activity, corneocyte “bricks” with their chemically resistant cornified cell envelopes, as well as complex interdigitations with neighboring corneocytes, forming a formidable physical barrier to pathogen ingress. In addition, the low water content and highly acidic surface pH (~5.0) of a nonoccluded (nonintertriginous) SC creates a hostile milieu for common pathogens, such as *S aureus*. At the same time, the acidic surface pH of a normal SC provides ideal growth conditions for normal cutaneous microflora, including both corynebacteria and Micrococaceae, such as *Staphylococcus epidermidis*.²⁶

Conclusions

Distinguishing the primary causes of allergic disorders from secondary host responses is challenging because of the multiplicity of factors that correlate with allergies. Two views continue to compete with each other, the “inside-outside” and the “outside-inside.”

In this review, we have focused on the role of the epidermal barrier function in the pathophysiology of AD. Specifically, we presented data in support of a barrier-initiated pathogenesis of AD, ie, the “outside-inside” concept.

In all fairness, despite very convincing evidence to support the barrier-initiated pathogenesis of AD, the view that AD reflects the downstream consequences of a primary immunologic abnormality cannot be dismissed out of hand. Almost every line of evidence in support of the role of the epidermal barrier as the “driver” of the disease activity can be challenged and at least partially contradicted by opposing evidence.

At the end of the day, however, although we cannot prove unequivocally that disruption of the epidermal barrier is the primary cause of AD, it is clear that this possibility must be taken into account. Further research in that direction is clearly warranted and highly recommended.

Until more data are available and until all the dust settles around this issue, we should take advantage of what we already know and use our knowledge for practical purposes. Deployment of specific strategies to restore the barrier function in AD means the use of moisturizers as first-line therapy. We strongly suspect that it will not be long until specific cytokines involved in barrier repair will enter the therapeutic scene as well.

References

- Wolff K, Goldsmith L, Katz S, et al. Fitzpatrick's Dermatology in General Medicine. New York: McGraw-Hill Companies, Inc.; 2008.
- Sandilands A, Sutherland C, Irvine AD, et al. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* 2009;122:1285-94.
- Rawlings AV, Harding CR. Moisturization and skin barrier function. *Dermatol Ther* 2004;17(Suppl 1):43-8.
- van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ* 2009;b2433:339.
- Rodriguez E, Baurecht H, Herberich E, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J Allergy Clin Immunol* 2009;123:1361-70.
- Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007;120:150-5.
- Irvine AD. Fleshing out filaggrin phenotypes. *J Invest Dermatol* 2007;127:504-7.
- O'Regan GM, Sandilands A, McLean WH, et al. Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 2008;122:689-93.
- Nemoto-Hasebe I, Akiyama M, Nomura T, et al. Clinical severity correlates with impaired barrier in filaggrin-related eczema. *J Invest Dermatol* 2009;129:682-9.
- Hubiche T, Ged C, Benard A, et al. Analysis of SPINK 5, KLK 7 and FLG genotypes in a French atopic dermatitis cohort. *Acta Derm Venereol* 2007;87:499-505.
- Eissa A, Diamandis EP. Human tissue kallikreins as promiscuous modulators of homeostatic skin barrier functions. *Biol Chem* 2008;389:669-80.
- Hachem JP, Houben E, Crumrine D, et al. Serine protease signaling of epidermal permeability barrier homeostasis. *J Invest Dermatol* 2006;126:2074-86.
- Man MQ, Barish GD, Schmuth M, et al. Deficiency of PPARbeta/delta in the epidermis results in defective cutaneous permeability barrier homeostasis and increased inflammation. *J Invest Dermatol* 2008;128:370-7.
- Sprecher E, Chavanas S, DiGiovanna JJ, et al. The spectrum of pathogenic mutations in SPINK5 in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. *J Invest Dermatol* 2001;117:179-87.
- Hachem JP, Wagberg F, Schmuth M, et al. Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. *J Invest Dermatol* 2006;126:1609-21.
- Walley AJ, Chavanas S, Moffatt MF, et al. Gene polymorphism in Netherton and common atopic disease. *Nat Genet* 2001;29:175-8.
- Nishio Y, Noguchi E, Shibasaki M, et al. Association between polymorphisms in the SPINK5 gene and atopic dermatitis in the Japanese. *Genes Immun* 2003;4:515-7.
- Kato A, Fukai K, Oiso N, et al. Association of SPINK5 gene polymorphisms with atopic dermatitis in the Japanese population. *Br J Dermatol* 2003;148:665-9.
- Weidinger S, Baurecht H, Wagenpfeil S, et al. Analysis of the individual and aggregate genetic contributions of previously identified serine peptidase inhibitor Kazal type 5 (SPINK5), kallikrein-related peptidase 7 (KLK7), and filaggrin (FLG) polymorphisms to eczema risk. *J Allergy Clin Immunol* 2008;122:560-8.
- Kusunoki T, Okafuji I, Yoshioka T, et al. SPINK5 polymorphism is associated with disease severity and food allergy in children with atopic dermatitis. *J Allergy Clin Immunol* 2005;115:636-8.
- Folster-Holst R, Stoll M, Koch WA, et al. Lack of association of SPINK5 polymorphisms with nonsyndromic atopic dermatitis in the population of Northern Germany. *Br J Dermatol* 2005;152:1365-7.
- Voegeli R, Rawlings AV, Breternitz M, et al. Increased stratum corneum serine protease activity in acute eczematous atopic skin. *Br J Dermatol* 2009;161:70-7.
- Bitoun E, Micheloni A, Lamant L, et al. LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome. *Hum Mol Genet* 2003;12:2417-30.
- Ong C, O'Toole EA, Ghali L, et al. LEKTI demonstrable by immunohistochemistry of the skin: a potential diagnostic skin test for Netherton syndrome. *Br J Dermatol* 2004;151:1253-7.
- Roedl D, Traidl-Hoffmann C, Ring J, et al. Serine protease inhibitor lymphoepithelial Kazal type-related inhibitor tends to be decreased in atopic dermatitis. *J Eur Acad Dermatol Venereol* 2009;23:1263-6.
- Elias PM. The skin barrier as an innate immune element. *Semin Immunopathol* 2007;29:3-14.
- Krien PM, Kermici M. Evidence for the existence of a self-regulated enzymatic process within the human stratum corneum—an unexpected role for urocanic acid. *J Invest Dermatol* 2000;115:414-20.
- Stehlik C. Multiple interleukin-1beta-converting enzymes contribute to inflammatory arthritis. *Arthritis Rheum* 2009;60:3524-30.
- McGirt LY, Beck LA. Innate immune defects in atopic dermatitis. *J Allergy Clin Immunol* 2006;118:202-8.
- Elias PM. Stratum corneum defensive functions: an integrated view. *J Invest Dermatol* 2005;125:183-200.
- Fluhr JW, Elias PM, Man MQ, et al. Is the filaggrin-histidine-urocanic acid pathway essential for stratum corneum acidification? *J Invest Dermatol* 2010;130:2141-4.
- Jeong SK, Kim HJ, Youm JK, et al. Mite and cockroach allergens activate protease-activated receptor 2 and delay epidermal permeability barrier recovery. *J Invest Dermatol* 2008;128:1930-9.
- Palmer CN, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
- Ong PY, Leung DY. The infectious aspects of atopic dermatitis. *Immunol Allergy Clin North Am* 2010;30:309-21.
- Bath-Hextall FJ, Birnie AJ, Ravenscroft JC, et al. Interventions to reduce *Staphylococcus aureus* in the management of atopic eczema: an updated Cochrane review. *Br J Dermatol* 2010;163:12-26.
- Nizet V, Ohtake T, Lauth X, et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* 2001;414:454-7.
- Yamasaki K, Gallo RL. Antimicrobial peptides in human skin disease. *Eur J Dermatol* 2008;18:11-21.
- Metz-Boutigue MH, Shooshtarizadeh P, Prevost G, et al. Antimicrobial peptides present in mammalian skin and gut are multifunctional defence molecules. *Curr Pharm Des* 2010;16:1024-39.

39. Ong PY, Ohtake T, Brandt C, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002;347:1151-60.
40. Goo J, Ji JH, Jeon H, et al. Expression of antimicrobial peptides such as LL-37 and hBD-2 in nonlesional skin of atopic individuals. *Pediatr Dermatol* 2010;27:341-8.
41. Harder J, Dressel S, Wittersheim M, et al. Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury. *J Invest Dermatol* 2010;130:1355-64.
42. Howell MD, Novak N, Bieber T, et al. Interleukin-10 downregulates anti-microbial peptide expression in atopic dermatitis. *J Invest Dermatol* 2005;125:738-45.