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The Role of Impaired Epidermal Barrier Function in Atopic Dermatitis

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ABSTRACT Atopic dermatitis (AD) is a chronic, inflammatory, pruritic skin disease with increasing prevalence. The etiopathogenesis of atopic dermatitis is multifactorial and involves a complex interplay of environmental and genetic factors that induce derangements in the structure and function of the epidermal barrier and immune system. Due to great heterogeneity of etiopathogenesis, there is also great variability of clinical presentation, and diagnosis can sometimes be challenging and difficult. Diagnosis mostly relies on clinical features and laboratory tests, but morphology alone cannot reliably establish the diagnosis, so the spectrum of features associated with AD must be considered. Traditionally, patients with AD have been separated into two different subgroups, i.e. intrinsic and extrinsic. Today, most of authors prefer the outside to inside and back to outside hypothesis, suggesting that the primary disorder lies in epidermal structure and function, resulting in inflammation and immunological downstream activation which further provokes secondary barrier abnormalities. In this review, we discuss the structure and function of the epidermal barrier and the role of impaired barrier function in etiopathogenesis of atopic dermatitis.

KEY WORDS: atopic dermatitis, skin barrier, atopic barrier, filaggrin, ceramides, *Staphylococcus aureus*, natural moisturizing factors

INTRODUCTION

Atopic dermatitis (AD) is a chronic, inflammatory, pruritic skin disease with increasing prevalence (affecting 15-30% of children and 2-10% of adults) (1-4). It is considered to be the first step in the "atopic march" that can progress to asthma (AA) and allergic rhinitis (AR) (5). Etiopathogenesis is multifactorial and involves a complex interplay of environmental and genetic factors that induce derangements in the structure and function of the epidermal barrier and immune system (6). Due to great heterogeneity of etiopathogenesis, there is also great variability of clinical presentation and diagnosis can sometimes be challenging and difficult (7). Diagnosis mostly relies on clinical features and laboratory tests, but morphology alone cannot reliably establish the diagnosis, and the spectrum of features associated with AD must be considered. Several sets of diagnostic criteria for AD have been proposed and validated; most clinician traditionally use Hanifin and Rajka's criteria, but full agreement amongst clinicians and uniformity of criteria are still lacking (8,9). Traditionally, patients with AD have been separated into two different

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subgroups, i.e. intrinsic and extrinsic. The extrinsic subtype presents with allergic sensitization to an external antigen with subsequent allergen-specific immunoglobulin E (IgE) production; the intrinsic variant is found in patients with all clinical features of AD but no detectable allergen-specific IgE. These subtypes may actually represent different stages of evolution based on the relative degree of sensitization. AD in infancy is thought to begin as "intrinsic"/non-allergic dermatitis, and over time it progresses to "true" atopy in the majority of cases *via* allergen exposure through what is being increasingly widely recognized as a primarily defective epidermal barrier function (6,10). Etiopathogenesis of AD is extremely complex, and several hypotheses have been advanced (6). Traditionally, it was thought that the primary pathogenic mechanism of AD was initiated by immune dysfunction leading to a T helper 2 (Th2) cytokine imbalance, increased inflammation, and secondary disruption of the epidermal barrier, usually referred as the inside to outside hypothesis (11). Recently, after very important findings on filaggrin (FLG) and its enormous impact on the structure and the function of the epidermal barrier, there is new emerging evidence suggesting that a primary defect in the stratum corneum (SC) plays a major role in driving the pathogenesis of AD, leading to sustained cytokine release, recruitment of pro-inflammatory molecules, and stimulation of a Th2 response. This is usually called the outside to inside hypothesis (11-13). Today, most authors prefer the outside to inside and back to outside hypothesis, suggesting that the primary disorder lies in epidermal structure and function, resulting in inflammation and immunological downstream activation which further provokes secondary barrier abnormalities (11,12).

EPIDERMAL BARRIER

The epidermal barrier (EB) is the first line of defense against the environmental influences and prevents water loss and preserves electrolyte balance. The epidermis generates a set of protective/defensive functions mediated by its unique differentiation end product, the stratum corneum (SC) (11). The permeability barrier resides in the SC, which is a multilayered tissue composed of flattened anucleate corneocytes surrounded by multiple lamellar sheets enriched in ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA), surrounded by a cell envelope composed of cross-linked proteins, the corneal envelope (14,15). Nucleated cells with cytoskeleton and tight and gap junctions contribute to the physical barrier. The permeability barrier retards transcutaneous water loss, microbial invasion, and entry of allergens and irritants. Another very important function is providing mechanical support (14).

The chemical barrier is formed by lipids, "acid mantle", antimicrobial peptides secreted by keratinocytes (KC), and the FLG protein that aggregates keratin filaments and produces natural moisturizing factors (NMF). The so called "acid mantle" is provided by free fatty acids (FFA), lactic acid from sweat, and urocanic acid, a degradation product of FLG (14). These components act together in ensuring normal keratinization and lipid synthesis, providing antimicrobial protection, and proper hydration of the skin (14). Hydrofobic lipids within the extracellular domain inhibit the outward movement of the water. Lipids are delivered, like their precursors, through secretion of the epidermal lamellar body (LB). As the SC is formed, these bodies deliver lipid constituents, lipid precursors, and enzymes required to generate CER and FFAs required for their organization into mature membrane structures (15). Additionally, proteases and their inhibitors derive from LB and orchestrate the orderly digestion of corneodesmosomes, allowing corneocytes to shed invisibly at the skin surface (15,16). The immunological barrier is closely linked to innate immunity of the skin. This results from complex interaction of the physical barrier, several immunologically active cell types, action of antimicrobial peptides, cytokines and encoded proteins called patern recognition receptors (PRR) (14,17). Cells involved in innate immunity are KCs, dendritic cells, Langerhans cells, neutrophils, and natural killer cells (17,18). Immune response and inflammation will be discussed further in the text.

BROAD BARRIER FAILURE IN ATOPIC DERMATITIS IS A DRIVER FOR CHRONICITY OF DISEASE

Dysfunction of the skin barrier in AD is the result of a complex interplay between the patient's genotype, environmental factors, and inflammation activity (11,12,19,20). Barrier abnormalities can be roughly characterized into inherited barrier abnormalities, the group of exogenous and endogenous stressors with further aggravation of barrier function, and impaired antimicrobial defense with further compromised barrier function (12). There is a complex interaction between genetic influences and exogenous and endogenous factors.

Recently, the focus has been on the role of genetic abnormalities which lead to key structural defects in EB, since 80-90% of all AD have a genetic background. The genes affected in AD include those encoding the FLG protein, serin proteases (SP), and protease inhibitors (12,14). Although mutations in FLG are best known, mutations in other members of the fused S-100 family of proteins (hornerin and filaggrin 2, the

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cornified envelope precursor (i.e. SPRR3), mattrin, which is encoded by TMEM79 and regulates the assembly of lamellar bodies, SPINK5, which encodes the serine protease inhibitor lymphoepithelial Kazal-type trypsin inhibitor type 1, and the fatty acid transport protein 4 have all been linked to AD (21). These abnormalities often only predispose to AD, and additional acquired stressors that further compromise barrier function, such as psychological stress, low ambient humidity, or high-pH surfactants are often required to trigger the disease (21). Th2 cytokines can also compromise barrier function by downregulating expression of multiple epidermal structural proteins, lipid synthetic enzymes, and antimicrobial peptides. All of these inherited and acquired abnormalities converge on the lamellar body secretory system, producing abnormalities in lipid composition, secretion, and/or extracellular lamellar membrane organization, as well as antimicrobial defense (21).

The mutation of the FLG gene *(FLG mut)* has the greatest influence, and today the majority of authors put it at the center of the etiopathogenesis. Filaggrin (FLG) was named by the late Peter Steinert, and its gene, among other proteins involved in terminal differentation, is located on chromosome 1q21, called the epidermal differentiation complex (13). The FLG gene *(FLG)* encodes for important multifunctional structural and functional protein of the epidermis. The name "filaggrin" is derived from the term "filament-aggregating protein". Loss-of-function mutations, in one or both alleles, result in reduced or completely absent levels of epidermal FLG (22,23). Loss of function mutations in *FLG* are the major risk factor for developing AD (24). Not all AD can be explained by FLG mutations however, since only 40% of patients with AD have this mutation, and not all individuals with *FLG mut* will develop AD (24-26). *FLG mut* alone does not suffice, and this fact is clearly shown in ichtyosis vulgaris where *FLG mut* is found in one or both alleles, but inflammation does not occur (12,27). Recent findings report a reduced skin barrier function irrespective of *FLG* genotype, suggesting the importance of additional factors for an impaired skin barrier in AD beside the impact of *FLG* mutation (28,29). About 10% of the general population in Northern Europe are heterozygotous mutation carriers, while 0.1% are homozygotous (12,30). Data for Southern Europe are scarce, but indicate that these mutations are rare or even absent (31-34). However, prevalence of *FLG mut* is much higher in patients with AD, and prevalence of this mutation is 25-50% in patients with AD in Northern Europe (24). Some authors view the north-to-south gradient in observed prevalence of *FLG mut* as evolutionary, speculating that these mutations have evolved due to low exposure to the sun and that this might be closely related to vitamin D in lightly pigmented Northerners. It is also known that NMF is responsible for UV protection. Another hypothesis and potential explanation is increased penetration of microorganisms as an *in vivo* mechanism of vaccination (13,35-37).

FLG loss-of-function mutations show population specificity (34). Since the discovery of the first two loss-of-function mutations (R501X and 2282del4) in 2006, these two mutations, along with the less prevalent S3247X and R2447X, have been studied and are present in 7-10% of the Caucasian population of Europe (24,35,41). More than 20 other rare or family-specific mutations have been discovered in white European ancestry (35). The population specificity indicates that they have arisen after one population has become split away from another (35). The Asian population has their own mutation spectra (35,38,39). The prevalence of *FLG* null-mutations varies across Europe, but R501X and 2282del4 are the two most common mutations and they have consistently shown significant association with AD across the continent, with the exception of the Italian population (32,33,35,40). Full sequencing of *FLG* in the Italian population identified only 3 additional mutations and no association with AD (32,33).

In those patients in whom AD is linked to FLG mutation, there is a genotype-phenotype correlation (42-44). Prevalent and low-frequency null-mutation in *FLG* is associated with early onset and persistent atopic eczema (42). FLG haploinsufficiency is highly penetrant and is associated with increased severity of eczema (43). The phenotype of early onset (before 2 years of age), persistent, and severe eczema has shown the strongest and most significant statistical association with the combined FLG null phenotype, with an odds ratio of up to 7.7 (44-46). However, the milder phenotype has also shown a statistically significant association (44,47). Beside loss-of-function mutation of *FLG,* there is another important genetic influence on FLG levels (12). The FLG gene is a repetitive gene and shows intragenic copy number variations (CNV), with the number of repeats between 10 to 12 (48). All reported *FLG* null-mutations are nonsense or frameshift mutations, resulting in truncation of the profilaggrin molecule. *FLG* encodes for profilaggrin (ProFLG), polymers released from keratohyalin granules of stratum granulosum and proteolitically cleaved into 10-12 identical FLG monomers by enzymes. The liberated FLG monomers aggregate keratin filaments into tight bundles, resulting in collapse and flattening of corneocytes. FLG and other epidermal differentiation-linked proteins are crosslinked

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into the cornified envelope by transglutaminases. Keratin 1 and 10 associate with FLG and its degradation products and constitute the bulk of cytosol of the anucleate corneocytes (KC) (12).

Besides structural function and mechanical strength, FLG has another very important function. As KCs move apically, FLG detaches from the cornified envelope, followed by degradation into constituent aminoacids, glutamine, arginine, and histidine and into their deiminated metabolites (12,49-51). Histidine is a substrate for histidase which generates trans-urocanic acid (tUCA), which is a major ultraviolet B (UVB)-absorbing epidermal chromophore that also contributes to the acid mantle of the skin (49). Glutamine is converted into pyrrolidone-5-carboxilic acid (PCA), a major component of "natural moisturizing factors" (NMF) of the SC and therefore a potent humectant, accounting for much water retention in the SC (49-51).

Another function of NMF is acidification, or, more precisely, maintaining proper pH values of the skin. Acidification is very closely connected to the antimicrobial function of the barrier as well as to proteolytic activity and release of proinflammatory cytokines (12,52). A loss of function mutation in *FLG* is strongly associated with an elevated pH of the SC surface, which might negatively influence SC lipid production, activity of various proteases involved in inflammatory processes, and desqumation and antimicrobial defense (12). Therefore, FLG deficiency affects epidermal protein expression and organization (12,53).

There is also evidence of environmental and inflammation-driven reductions in levels of epidermal FLG, and not only due to genetic reasons (12). It is known that monomeric FLG is hydrolyzed in a humidity-sensitive fashion as environmental humidity declines, following a rapid shift from a humid to a dry environment (54). Children with AD linked to *FLG mut* more frequently have dermatitis on air-exposed areas when compared to the wild type (55). UVB radiation is another climatic and environmental influence, single dose of UVB exposure leads to downregulation of *FLG* expression (12,56). Beside climatic influence, exposure to water and irritants also reduces FLG levels (57). Mechanical disturbance of the barrier, such as scratching as a result of intense pruritus in AD, also downregulates FLG expression. Moreover, further barrier disruption in the chronic stages of AD through mechanical scratching not only perpetuates, but alters the response to a mostly Th1 type (12). Topical therapy can also have an impact on the expression of epidermal FLG. It is interesting that prolonged use of topical corticosteroids, but not topical tacrolimus, reduces epidermal FLG (21,58,59).

Other genes can be affected and have important influence on epidermal barrier, such as genes that upregulate serin proteases (SPs). Genetic defects due to gain of function mutations in the kalikrein 7 gene encoding serin proteases up-regulate serin proteases (SPs) (14). Increased activity of SPs degrades desmosomes, lipid-processing enzymes which results in decreased production of ceramides (12). It also activates the protease activator type 2 receptor (PAR-2) by direct cleavage and inducing its signal cascade, resulting in downregulation of lamellar body secretion and increased production of interleukin 1 alpha (IL-1α) and interleukin 1 beta (IL-1β) from corneocytes leading to inflammation (60,61). Protease inhibitors (PIs) are another important part of the balance due to the fact that these inhibitors inhibit the function of proteases (14). Cystatin A is a cystein PI secreted by sweat glands to inhibit the degradation by exogenous (microbial) proteases. Gene mutations for this PI has been found in patients with AD (14,19,60,62). The best example of impaired protease inhibitor function is in Netherthon syndrome, which is characterized with severe eczema, mucosal atopy, and severe food allergies. This syndrome is linked to loss-of-function mutation in gene SPINK-5 which encodes for LEKTI (lymphepithelial Kazal type inhibitor), which is also a protease inhibitor (14,63,64).

INFLAMMATION AND THE ROLE OF IMMUNE SYSTEM

Inflammation is another independent factor that reduces levels of the FLG and NMF (12). This occurs independently of *FLG mut* status (65,66). Inflammation results from increased SPs activity, which activates the primary cytokines, IL-1 α, and IL-1β from their proforms stored in KCs (20). Increased protease activity is the result of elevated pH, and this is typically the first step in a cascade of inflammation (65-68). The second step is sustained antigen ingress through the defective barrier, leading to Th2-dominant infiltrate (11). Certain antimicrobial antigens are preferentially associated with AD and show a propensity to trigger AD, especially in FLG-deficient patients (21). These microbial antigens activate SP activity and further damage the barrier (more details in the section on antimicrobial function).

Despite current accumulating evidence on barrier-initiated pathogenesis of AD, there is evidence on other mechanisms whereby Th2 generated cytokines worsen AD (11,20,65,70). The key Th2 cytokines, IL-4, and IL-13 cause further aggravation of AD by causing the following negative effects: IL-4 causes inhibition of ceramide synthesis, inhibition of KC-differentia-

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tion-linked proteins (loricrin and FLG), and decreased desmoglein-3 expression with compromised SC integrity (65,70-72).

The skin is immunologically active and the skin barrier has an immunological function. One of the key elements in the immunological barrier are PRRs. They can be transmembrane/intracellular receptors and include a group of toll-like receptors (TLR), through which KCs sense microbial invasion and injury (13,14,73). Activation of TLRs on KCs can upregulate proinflammatory cytokine expression via the activation of NF-κB (73). Cells secrete cytokines, such as TNF-α, and interleukins such as IL-6 and IL-1α, which results in stimulation of cell proliferation and lipid synthesis (13,14,73). It is interesting that acute barrier disruption causes an increase in cytokines crucial for barrier repair, but chronic disruption, on the other hand, leads to inflammation and epidermal proliferation (13,14).

Although the role of the innate immune system has not yet been elucidated, it is known that activation of innate immune receptors results in the release of various inflammatory mediators derived from KCs which probably decrease FLG expression (75,76). Therefore, inflammation causes decreased FLG expression, regardless of *FLG* mutation status.

One of the hallmarks of the extrinsic variant of AD is elevated serum IgE. Serum IgE acts as an autoantibody, but it is still not known whether it targets FLG as well; it is however known that IgE autoreacts with a variety of KC-antigens (77). Autoantigens are released from damaged tissue triggered by mechanical injury/scratching (78).

It recently was discovered that the gene MAP-17 is associated with the T-helper cell cytokine-induced down regulation of FLG transcription in human KCs (79). Various cytokines such as IL-4, IL-17, IL-22, IL-25, and IL-31 can upregulate the MAP-17 gene, which further down-regulates FLG synthesis, CER production, and cell-to-cell adhesion (80-84). This provides a link between altered FLG and lipids in patients with AD.

ROLE OF LIPIDS

The structure of the barrier is closely related to its function. FLG has an enormous impact on the barrier, but a second very important cause of barrier alteration is change in the structure and composition of SC lipids. The permeability barrier resides in the SC which is multilayered tissue composed of flattened anucleate corneocytes surrounded by multiple lamellae sheets enriched in ceramides (CER), cholesterol (CHOL), and free fatty acids (FFA) (15). Hydrofobic lipids within the extracellular domain have a significant role because they inhibit the outward movement of the water (15). Lipids are delivered as their precursors through secretion of the epidermal lamellar body (LB). As the SC is formed, LB deliver lipid constituents, lipid precursors, and enzymes required to generate CER and FFAs that are required for their organization into mature membrane structures (15). Additionally, proteases and their inhibitors derive from LB and orchestrate the orderly digestion of corneodesmosomes, allowing corneocytes to shed invisibly at the skin surface (15,16,85). CER are the main components of the multilayered lamellar bilayers between the corneocytes and thus the key factor in water retention and overall integrity of the barrier (86). It is well known that CER, with CHOL and FFA in a ratio of 3:1:1, are the most prominent group of physiological lipids and show alterations in skin of patients with AD. Different classes of CER differ in the length of the free fatty acid chain and sfingosin base. To date, 12 CER subclasses have been identified in humans, showing a wide chain length distribution (87-89). CER play a crucial role in the lipid organization due to their characteristic molecular architecture (90). In healthy SC, lipids form two lamellar phases with repeat distances of 6 nm and 13 nm, called the short periodicity phase and long periodicity phase (91). Lamellar lipids have a dense orthorhombic lateral organization, although a subpopulation of lipids can be densely packed in a hexagonal organization (92,93). Several studies on patients with AD reported reduction in total ceramids and reduction in long-chain CER and FFA (94-97). This substitution of shorter chain FFA results in lipid disorganization that likely further compromises barrier function (87,98). The relative chain length of CER is shorter and this correlates with an altered lipid organization and barrier function. This effect is *FLG mut* independent, but is associated with the levels of NMF (87). This fact suggests that *FLG* (genotype), NMF (phenotype), and other (translational and environmental) factors can influence NMF levels (87). Copy number variation of *FLG* and interleukin levels can probably downregulate FLG expression (70). NMF levels lead to elevated pH levels which may affect enzymes involved in CER synthesis and proteolytic activity (87,99-101). Changes in lipid composition and organization are observed in lesional skin, but also in nonlesional skin where increased levels of extremely short C34 chains were found. It is also reported that CER chains length is an important determinant of lateral lipid organization (87,97). These changes in CER chain length distribution correlate with changes in lipid organization, skin barrier function, disease severity, and levels of NMF. Therefore, chain length of CER is an important factor in skin barrier dysfunction. It is

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suggested that this increase in short chains is due to a misbalance in the activity of some of the members of the elongase family, since the elongase family plays an important role in the elongation of fatty acids in the epidermis (98). Some other studies showed that the CER composition and chain length, rather than the ratio between lipid classes, plays a major role in the increased transepidermal water loss (TEWL) in nonlesional skin (102).

According to some authors, FLG deficiency also affects the delivery of lipids (12). Due to cytoskeletal abnormalities, loading into LB is partially compromised, resulting in some KCs being deficient in LB (103). This could reduce the normal quantities and organization of the extracellular lamellar bilayers (104). This provides a link between FLG and lipid composition of the epidermis.

Th2 cytokines can also compromise barrier function by downregulating expression of multiple epidermal structural proteins, lipid synthetic enzymes, and antimicrobial peptides (68). All of these inherited and acquired abnormalities converge on the lamellar body secretory system, producing abnormalities in lipid composition, secretion, and/or extracellular lamellar membrane organization, as well as antimicrobial defense (68). It has been recently shown that mutations in the genes FATP4 (encodes for FATP4- fatty acid transporter in the suprabasal layer of epidermis) and Tmem79 (encodes for a component in the lamelar granule secretory system) both associate with an increased risk of AD. These are both independent of *FLG mut* and can emphasize the importance of altered lipid production and secretion in the pathogenesis of AD (12,105,106).

Inflammation is another significant factor that can influence lipid status (12). In the chronic phase of AD, γ-IFN down-regulates two fatty acid elongases (ELOVL1 and ELOVL4) required to generate the very long chain N-acyl fatty acids in CER and FFA (98). In this way, inflammation negatively influences lipid composition.

pH OF THE BARRIER AND PROTEASE ACTIVITY

Maintenance of adequate pH is very important for adequate skin barrier and function by preventing unnecessary activation of serin proteases (85,99,101). As already mentioned, reduction of the epidermal FLG results in reduction of its NMF, which is responsible for maintaining acidic pH. There is an allele dose-dependent effect, and homozigotous *FLG mut* carriers usually have the highest pH levels (50,51). There is a compensatory mechanism due to upregulation of Na+/H+ antiporter (NHE-1) which seems to counteract this pH increase, which may explain why elevated pH is not always seen in all *FLG mut* carriers (107). pH-sensitive proteases are activated following the pH increase (12). This causes premature degradation of corneodesmosomes and compromises intercellular connections and activates IL-1α and IL-1β (108,109). The most compelling evidence comes from Netherthon syndrome (12,108). Protease activation impairs CER production and likely stimulates Th2 inflammation in AD (12). In addition to the protease effect of kalikreins, they also bind to protease activated receptors type 2 (PARS2) which downregulate lamellar body secretion and initiate a cascade of innate inflammatory responses, including activation of thymic stromal lymphoprotein (TSLP) from KCs. TSLP is a masterswitch for initiation of Th2 inflammatory responses (12,108,110,111). TSLP is produced by KCs, and secretion is induced by exposure to allergens, microorganisms, or mechanical injury (111-113). TSLP has an important role in the etiopathogenesis of itch, since it induces cutaneous nerves to release neuropeptides causing itch, with the consequence of scratching and further damage to the barrier and secondary superinfection (114).

INCREASED ALLERGEN PENETRATION AND ENHANCED IMMUNE REACTIVITY

FLG deficiency permits increased allergen penetration and enhances immune reactivity (12). FLG-null mouse is a model for ichtiosis vulgaris without AD, and these mice show accelerated allergen penetration through the SC as well as enhanced contact hypersensitivity response (115,116). In such cases, double allele mutations in FLG cause inherited skin barrier dysfunction (106). Another piece of evidence comes from the "flaky-tail" mouse with matted mutation (Tmem 79) which shows spontaneous inflammation and also increased penetration of allergens as well as a low threshold to irritation to allergens and skin irritants (116). Allergic sensitization and inflammation causes further exacerbation of the barrier deficit, which can be observed in the elevation of TEWL. Exposure to allergens or other stressors activates the inflammasome, protease, and downstream immune mechanisms (12). Some microorganisms can secrete and activate serine proteases and cause proteolitic damage to the barrier, but also cause release of active forms of IL-1α and IL-1β from KCs and initiate inflammation (117,118). Additionally, in conditions of FLG deficiency, stimulation of TLR 3 enhances production of TSLP (119,120). These findings suggest a link between FLG deficiency, Th2 promotion, and systemic disease (12,120). These observations on mouse mod-

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els are yet to be confirmed in humans, but results from metaanalysis done in 2009 show that patients with AD and with FLG mutations have increased risk of sensitization to allergens and development of asthma, allergic rhinitis, and food allergy (121).

ANTIMICROBIAL DEFENCE

Another important function of the skin barrier is the antimicrobial function, which strongly encourages the growth of nonpatogenic flora (122). Healthy skin is colonized by large numbers of microorganisms, which are classified into resident and transient flora (123). Resident flora colonizes the skin in proportionally constant numbers and includes coagulase-negative staphylococci (*S. epidermidis, S. hemolyticus, S. hominis*). Transient flora is caused by contact of the skin with external objects. *Staphyloccocus aureus* (SA) is not a member of resident flora (123). It has prevalence of approximately 5% in healthy population (124). More than 90% of patients with AD have SA on their lesional, and 30-100% on nonlesional skin (125,126). Permeability and antimicrobial dysfunction are well-known features of AD and are co-regulated and interdependent (19,61,67,68,127). Failure of the permeability barrier favors secondary infection, and secondary infection further compromises the barrier (19). Again, FLG plays an important role (12). FLG seems to protect against colonization of certain microorganism (12). *Staphylococcus aureus* (SA) has an important role in etiopathogenesis of AD. The skin of patients with AD exhibits a striking susceptibility to colonization and infection with SA (123). Patients with AD have the highest counts of SA on lesional skin, but often colony counts are elevated on even nonlesional/clinically normal skin (128). There is an extreme difference between lesional and nonlesional skin, and these counts differ by 100 to 1000 times (123,129). Indeed, SA colonization is both a cause and a consequence of allergic skin inflammation (123). Impaired barrier in AD predisposes to pathogen colonization not only due to increased pH, but also because of reduced levels of FFA and ceramide metabolite sfingozine which exhibits potent antimicrobial activity (19, 130,131). Surface proteins on SA downregulate epidermal FFA production, therefore aggravating permeability and antimicrobial function (132). Adherence of SA is increased in patients with AD due to injury of the skin barrier which leads to exposure of extracellular matrix adhesins for SA, epidermal and dermal laminin, and fibronectin (133,134). Fibronectin in combination with plasma exudation of fibrinogen allows SA to bind to the skin (133,134). Scratching further damages the barrier and causes cytokine release with upregulation of the expression of adhesins (135). There is evidence that Th2 inflammation, or, more precisely, IL-4 plays important role in the enhancement of SA binding by inducing the production of fibronectin (134,136). Alteration of lipid composition is another contributing factor due to decrease in ceramides which leads to dry and cracked skin and predisposes to colonization (137). SA stimulates hydrolysis of CER by bacteria-produced ceramidases (138). Sphingosine exerts a potent antimicrobial effect in normal conditions, but, as a result of reduced activities of acid ceramidases and decreased levels of CER in general, the levels of sphingosine are also reduced (139). Normal FLG expression increases sphingomyelinase secretion (140).

Another important reason for SA colonization is defective innate immune response (122,123,132). There are 2 classes of endogenous antimicrobial petides; β-defensins and cathelicidins, produced by KCs with antimicrobial effects against bacterial, fungal, and viral antigens (123). Antimicrobial peptides are delivered to SC intercellular domains via secretion of LB contents (19,141-143). Some of these antimicrobial peptides are constitutionally produced, while others (HBD-2, LL-37) are induced by TNF-α following skin inflammation or injury (123). These endogenous antimicrobial peptides are essential for defending the skin against bacterial infection (144). Members of two key families of antimicrobial proteins, the human cathelicidin product (hCAP), LL-37, and human β-defensins (hBD) 2 and 3 are downregulated in Th-2-dependent fashion (145-147). Fragments of hCAP and hBD3 exhibit potent antistaphylococcal activity (147).

In 70% of all patients with AD, non-toxin producing-SA strains are replaced with enterotoxin-producing strains and exacerbate AD via 3 mechanisms: these strains cause more frequent clinically apparent reactions; some toxins stimulate pruritus and production of specific IgE; and finally, some toxins act as superantigens by stimulating T- and B-cell production and switching to allergen-specific or superantigens that stimulate IgE production (19,66,122,123,148). Staphylococcal superantigens may directly stimulate antigen presenting cells and KCs to produce several proinflammatory cytokines, such as IL-1, TNF-α, and IL-12 and contribute to persistence and exacerbation of allergic skin inflammation (123,149). They recognize and stimulate T cells bearing the specific TCRβ which results in massive activation of polyclonal T cells (up to 20% of total T cell population) (123). Superantigen-producing SA strains further exacerbate AD through augmentation of IgE production and development of specific IgE against SA exotoxins (122). It has been reported that T cells stimulated with superantigens become resistant to immunosuppressive effect of

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corticosteroids (123,150). Activated T cells produce IL-31 which induces pruritus. It is well known fact that infections (such as folliculitis) are also extremely pruritic. Pruritus starts an itch-scratch cycle which causes further damages to the barrier and facilitates spreading of the infection (122). Another very interesting observation is that SA-α toxin preferentially destroys FLG-deficient KCs, so FLG again plays an important role (140). FLG breakdown products reduce growth rates of SA (151). In general, patients with AD with *FLG mut* have 7-fold increased risk of bacterial infection when compared to FLG wild type patients (12,152). Some microbes such as SA, dust mites, and scabies induce proteolitic activity with cleaving corneodesmosomal proteins and FLG, contributing further to the cycle of inflammation and pruritus.

Patients with AD are also prone to viral infections, especially to herpes simplex (7,153). Eczema herpeticatum (EH) is clinically defined as disseminated herpes simplex viruses (HSV) infection which is almost exclusively seen in patients with AD (154). Nectin-1 is an entry receptor for HSV (155). Nectin-1, a cell adhesion molecule belonging to the immunoglobulin superfamily, can bind to virion glycoprotein D (gD) to mediate entry of HSV. Nectin-1 colocalizes with E-cadherin at adherens junctions in epithelial cells. The disruption of cell junctions can result in the redistribution of nectin-1 (155). Lack of plasmacytoid dendritic cells, cathelicidin production, and an abnormal IFN-γ response to HSV all contribute to pathogenesis of eczema herpeticatum (156-158). The cathelicidin peptide LL-37 possesses antiviral activity against HSV and demonstrates the importance of variable skin expression of cathelicidins in controlling susceptibility to eczema herpeticum (156). Additionally, serum IgE levels might be a surrogate marker for innate immune function and serve as a biomarker for determining which patients with AD are susceptible eczema herpeticatum (156).

Hinz *et al*. showed that patients with AD, recurrent HSV infection, and a history of eczema herpeticatum had higher serum IgE levels, more severe AD, and a higher IgE sensitization profile when compared to a group without history of eczema herpeticatum (159). It is interesting to point out that staphylococcal α-toxin increases the HSV load and therefore contributes to EH (160). A subset of patients with AD is prone to disseminated herpes simplex virus (HSV) infection, and biomarkers that identify this subgroup are lacking. A recent study has found that the two master regulators of innate immune responses IRF3 and IRF7 are suppressed in this subgroup, suggesting that abnormalities of up-stream of IRF3 and IRF7 signal pathways (161).

CONLUSIONS

FLG has the central role in the etiopatogenesis of AD, since FLG deficiency affects epidermal protein expression and organization. A loss of function mutation in *FLG* is strongly associated with an elevated pH of the SC surface which might negatively influence SC-lipid production, activity of various proteases involved in inflammatory processes, and desquamation and antimicrobial defense. Not all patients can attribute their AD to FLG mutations. Up to 40% of patients with AD have one or two FLG mutations, and these mutations are associated with early onset as well as more severe and prolonged disease. Other patients with no FLG mutations have other causes of impaired barrier. Lipids are another very important determinant of skin barrier. Altered composition and structure of the lipids has a great impact on the function of the barrier. Since many proteolytic enzymes and protease inhibitors are involved in obtaining the normal function and structure of the barrier, there are numerous factors which can be influenced and whose function can be altered. All important functions of the barrier are a result of the barrier's structure and organization. Except genetic predisposition, where FLG mutation has the greatest influence, a many exogenous and endogenous stressors can further compromise the barrier. All traditional, "gold standard" therapeutic options today aim at inflammation and modulation of inflammatory response. We now know that everyday skin care and use of emollients is extremely important. What kind of emollients patients use is also very important, since every local therapy has an impact on the barrier. Since the barrier is impaired in patients with AD, some local products can even worsen and further compromise the barrier. Understanding the structure of the barrier and complexity of the function is a first step in understanding the etiopathogenesis of AD. It seems that every patient has AD with their own, unique set of mutations of important structural proteins, lipids, and proteolitic enzymes and their inhibitors, so it is probably wise to determine what kind of impairment of the barrier patient has and use this information in the therapeutic approach. It would be also advisable to take this into consideration in everyday life beginning with birth, since most of authors today the barrier impairment to be the first step in etiopathogenesis, with numerous genetic and environmental impacts. It is likely that in future we will apply the lessons from the barrier to create a more complex therapeutic approach. Corticosteroids and immunomodulators have an impact only on immunological response, but do not influence the barrier impairment. The barrier is also influenced by psychosociological stress and numer-

ous exogenous stressors that are common in modern life, such as environmental pollution and numerous hygiene products which cause further damage to the barrier. The rational approach would be aiming at reduction of consequences caused by genetics, such as FLG mutation and FLG deficiency, since epidermal FLG level is not only determined by the mutation itself. Except the genetic influence, another important determinant are numerous endogenous and exogenous stressors which further diminish barrier function. Local therapy can help in breaking the scratch-itch cycle and inflammation, since scratching causes mechanical damage to the barrier with the consequence of inflammatory response driven by damaged keratinocytes. In patients who show lipid aberration, a wise approach would be aiming at normalizing ceramide chain length distribution. Recently, much effort has been made to understand the role of the emollients, since their composition and characteristics are a very important determinant that can help improve impairment of the barrier.

References:

1. Cookson WO, Ubhi B, Lawrence R, Abecasis GR, Walley AJ, Cox HE, *et al*. Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. Nat Genet 2001;27:372-3.
2. Shaw TE, Currie GP, Koudelka CW, Simpson EL. Eczema prevalence in the United States: data from the 2003 National Survey of Children's Health. J Invest Dermatol 2011;131:67-73.
3. Kay J, Gawkrodger DJ, Mortimer MJ, Jaron AG. The prevalence of childhood atopic eczema in a general population. J Am Acad Dermatol 1994;30:35-9.
4. Shamssain M. Trends in the prevalence and severity of asthma, rhinitis and atopic eczema in 6- to 7- and 13- to 14-yr-old children from the north-east of England. Peadiatr Allergy Immunol 2007;18:149-53.
5. Zheng T, Yu J, Oh MH, Zhu Z. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. Allergy Asthma Immunol Res 2011;3:67-73.
6. Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. N Engl J Med 2011;365:1315-27.
7. Wollenberg A, Seba A, Antal AS. Immunological and molecular targets of atopic dermatitis treatment. Br J Dermatol 2014;170:7-11.
8. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 1993;186:23-31.
9. Samochocki Z, Dejewska J. A comparison of criteria for diagnosis of atopic dermatitis in children. World J Pediatr 2012 8:355-8.
10. Roguedas-Contios AM, Misery L. What is intrinsic atopic dermatitis? Clin Rev Allergy Immunol 2011;41:233-6.
11. Elias PM, Hatano Y, Williams ML. Basis for the barrier abnormality in atopic dermatitis: outside-inside-outside pathogenic mechanisms. J Allergy Clin Immunol 2008;121:1337-43.
12. Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. J Allergy Clin Immunol 2014;134:792-9.
13. Irvine AD, McLean WH. Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. J Invest Dermatol 2006;126:1200-2.
14. Thawer-Esmail F. Skin barrier function and atopic eczema. Curr Allergy Clin Immunol 2011;24:193-8.
15. Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. Adv Lipid Res 1991;24:1-26.
16. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, *et al*. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. J Invest Dermatol 2004;122:1235-44.
17. De Benedetto A, Agnihothri R, McGirt LY, Bankova LG, Beck LA. Atopic dermatitis: a disease caused by innate immune defects? J Invest Dermatol 2009;129:14-30.
18. Ladd M, Sharma A, Huang Q, Wang AY, Xu L, Genowati I, *et al*. Natural killer T cells constitutively expressing the interleukin-2 receptor α chain early in life are primed to respond to lower antigenic stimulation. Immunology 2010;131:289-99.
19. Elias PM. Skin barrier function. Curr Allergy Asthma Rep 2008;8:299-305.
20. Elias PM. Therapeutic implications of a barrier-based pathogenesis of atopic dermatitis. Ann Dermatol 2010;22:245-54.
21. Bisgaard H, Simpson A, Palmer CN, Bønnelykke K, McLean I, Mukhopadhyay S,*et al*. Gene-environment interaction in the onset of eczema in infancy: filaggrin loss-of-function mutations enhanced by neonatal cat exposure. PloS Med, 2008;5:e131.
22. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, *et al*. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006;38:337-42.

23. Kezic S, O'Regan GM, Yau N, Sandilands A, Chen H, Campbell LE, *et al*. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. Allergy 2011;66:934-40.
24. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, *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.
25. O'Regan GM, Sandilands A, McLean WH, Irvine AD. Filaggrin in atopic dermatitis. J Allergy Clin Immunol 2008;122:689-93.
26. Irvine AD. Fleshing out filaggrin phenotypes. J Invest Dermatol 2007;127:504-7.
27. Gruber R, Elias PM., Crumrine D, Lin TK, Brandner JM, Hachem JP, *et al*. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. Am J Pathol 2011;178:2252-63.
28. Jakasa I, Koster ES, Calkoen F, McLean WH, Campbell LE, Bos JD, *et al*. Skin barrier function in healthy subjects and patients with atopic dermatitis in relation to filaggrin loss-of-function mutations. J Invest Dermatol 2011;131:540-2.
29. Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ, *et al*. Lamellar lipid organization and ceramide composition in the stratum corneum of patients with atopic eczema. J Invest Dermatol 2011;131:2136-8.
30. Thyssen JP, Bikle DD, Elias PM. Evidence that loss-of-function *filaggrin* gene mutations evolved in northern europeans to favor intracutaneous vitamin D3 production. Evol Biol 2014;41:388-396.
31. Ponińska J, Samoliński B, Tomaszewska A, Raciborski F, Samel-Kowalik P, Walkiewicz A. Filaggrin gene defects are independent risk factors for atopic asthma in a Polish population: a study in ECAP cohort. PLoS One 2011 Feb 18;6:e16933.
32. Cascella R, Foti Cuzzola V, Lepre T, Galli E, Moschese V, Chini L, *et al*. Full sequencing of the FLG gene in Italian patients with atopic eczema: evidence of new mutations, but lack of an association. J Invest Dermatol 2011;131:982-4.
33. Giardina E, Paolillo N, Sinibaldi C, Novelli G. R501X and 2282del4 filaggrin mutations do not confer susceptibility to psoriasis and atopic dermatitis in Italian patients. Dermatology 2008;216:83-4.
34. Sabolić Pipinić I, Varnai VM, Turk R, Breljak D, Kezić S, Macan J. Low frequency of filaggrin null mutations in Croatia and their relation with allergic diseases. Int J Immunogenet 2013;40:192-8.
35. Brown SJ, McLean WH. One remarkable molecule: filaggrin. J Invest Dermatol 2012;132:751-62.
36. Elias PM, Williams ML. Re-appraisal of current theories for the development and loss of epidermal pigmentation in hominins and modern humans. J Hum Evol 2013;64:687-92.
37. Thyssen JP, Thuesen B, Huth C, Standl M, Carson CG, Heinrich J, *et al*. Skin barrier abnormality caused by filaggrin (FLG) mutations is associated with increased serum 25-hydroxyvitamin D concentrations. J Allergy Clin Immunol 2012;130:1204-7.
38. Enomoto H, Hirata K, Otsuka K, Kawai T, Takahashi T, Hirota T, *et al*. Filaggrin null mutations are associated with atopic dermatitis and elevated levels of IgE in the Japanese population: a family and case-control study. J Hum Genet 2008;53:615-21.
39. Zhang H, Guo Y, Wang W, Shi M, Chen X, Yao Z. Mutations in the filaggrin gene in Han Chinese patients with atopic dermatitis. Allergy 2011;66:420-7.
40. Rodríguez E, Baurecht H, Herberich E, Wagenpfeil S, Brown SJ, Cordell HJ, *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.
41. Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, *et al*. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet 2007;39:650-4.
42. Brown SJ, Sandilands A, Zhao Y, Liao H, Relton CL, Meggitt SJ, *et al*. Prevalent and low-frequency null mutations in the filaggrin gene are associated with early-onset and persistent atopic eczema. J Invest Dermatol 2008;128:1591-4.
43. Brown SJ, Relton CL, Liao H, Zhao Y, Sandilands A, McLean WH, *et al*. Filaggrin haploinsufficiency is highly penetrant and is associated with increased severity of eczema: further delineation of the skin phenotype in a prospective epidemiological study of 792 school children. Br J Dermatol 2009;161:884-9.
44. Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. J Invest Dermatol 2009;129:543-52.
45. Barker JN, Palmer CN, Zhao Y, Liao H, Hull PR, Lee SP, *et al*. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol 2007;127:564-7.
46. Weidinger S, Rodríguez E, Stahl C, Wagenpfeil S,

Klopp N, Illig T, *et al*. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J Invest Dermatol 2007;127:724-6.

47. Brown SJ, Relton CL, Liao H, Zhao Y, Sandilands A, Wilson IJ, *et al*. Filaggrin null mutations and childhood atopic eczema: a population-based case-control study. J Allergy Clin Immunol 2008;121:940-6.
48. Brown SJ, Kroboth K, Sandilands A, Campbell LE, Pohler E, Kezic S, *et al*. Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect. J Invest Dermatol 2012;132:98-104.
49. Harding CR, Aho S, Bosko CA. Filaggrin - revisited. Int J Cosmet Sci 2013;35:412-23.
50. Kezic S, O'Regan GM, Yau N, Sandilands A, Chen H, Campbell LE, *et al*. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. Allergy 2011;66:934-40.
51. Kezic S, Kemperman PM, Koster ES, de Jongh CM, Thio HB, Campbell LE, *et al*. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. J Invest Dermatol 2008;128:2117-9.
52. Contassot E, Beer HD, French LE. Interleukin-1, inflammasomes, autoinflammation and the skin. Swiss Med Wkly 2012 May 31;142:w13590.
53. Pendaries V, Malaisse J, Pellerin L, Le Lamer M, Nachat R, Kezic S, *et al*. Knockdown of filaggrin in a three-dimensional reconstructed human epidermis impairs keratinocyte differentiation. J Invest Dermatol 2014;134:2938-46.
54. Katagiri C, Sato J, Nomura J, Denda M. Changes in environmental humidity affect the water-holding property of the stratum corneum and its free amino acid content, and the expression of filaggrin in the epidermis of hairless mice. J Dermatol Sci 2003;31:29-35.
55. Carson CG, Rasmussen MA, Thyssen JP, Menné T, Bisgaard H. Clinical presentation of atopic dermatitis by filaggrin gene mutation status during the first 7 years of life in a prospective cohort study. PLoS One 2012;7:e48678.
56. Bernerd F, Asselineau D. Successive alteration and recovery of epidermal differentiation and morphogenesis after specific UVB-damages in skin reconstructed in vitro. Dev Biol 1997;183:123-38.
57. Törmä H, Lindberg M, Berne B. Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin in vivo. J Invest Dermatol 2008;128:1212-9.
58. Danby SG, Cork MJ. The effects of pimecrolimus on the innate immune response in atopic dermatitis. Br J Dermatol 2013;168:235-6.
59. Sheu HM, Tai CL, Kuo KW, Yu HS, Chai CY. Modulation of epidermal terminal differentiation in patients after long-term topical corticosteroids. J Dermatol 1991;18:454-64.
60. Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, *et al*. Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol 2009;129:1892-908.
61. Elias PM, Steinhoff M. "Outside-to-inside" (and now back to "outside") pathogenic mechanisms in atopic dermatitis. J Invest Dermatol 2008:128:1067-70.
62. Hatano Y, Man MQ, Uchida Y, Crumrine D, Scharschmidt TC, Kim EG, *et al*. Maintenance of an acidic stratum corneum prevents emergence of murine atopic dermatitis. J Invest Dermatol 2009;129:1824-35.
63. Hannula-Jouppi K, Laasanen SL, Heikkilä H, Tuomiranta M, Tuomi ML, Hilvo S, *et al*. IgE allergen component-based profiling and atopic manifestations in patients with Netherton syndrome. J Allergy Clin Immunol 2014;134:985-8.
64. Furio L, Hovnanian A. Netherton syndrome: defective kallikrein inhibition in the skin leads to skin inflammation and allergy. Biol Chem 2014;395:945-58.
65. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, *et al*. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol 2009;124(3 Suppl 2):R7-R12.
66. Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Méchin MC, *et al*. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. J Allergy Clin Immunol 2013;131:1094-102.
67. Elias PM, Wood LC, Feingold KR. Epidermal pathogenesis of inflammatory dermatoses. Am J Contact Dermat 1999;10:119-26.
68. Elias PM, Feingold KR. Does the tail wag the dog? Role of the barrier in the pathogenesis of inflammatory dermatoses and therapeutic implications. Arch Dermatol 2001;137:1079-81.
69. Kao JS, Fluhr JW, Man MQ, Fowler AJ, Hachem JP, Crumrine D, *et al*. Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of epidermal lipid synthesis accounts

for functional abnormalities. J Invest Dermatol 2003;120:456-64.

70. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, Debenedetto A. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol 2007;120:150-5.
71. Hatano Y, Terashi H, Arakawa S, Katagiri K. Interleukin-4 suppresses the enhancement of ceramide synthesis and cutaneous permeability barrier functions induced by tumor necrosis factor-alpha and interferon-gamma in human epidermis. J Invest Dermatol 2005;124:786-92.
72. Kurahashi R, Hatano Y, Katagiri K. IL-4 suppresses the recovery of cutaneous permeability barrier functions in vivo. J Invest Dermatol 2008;128:1329-31.
73. Panzer R, Blobel C, Fölster-Holst R, Proksch E. TLR2 and TLR4 expression in atopic dermatitis, contact dermatitis and psoriasis. Exp Dermatol 2014;23:364-6.
74. Takai T, Chen X, Xie Y, Vu AT, Le TA, Kinoshita H, *et al*. TSLP expression induced via Toll-like receptor pathways in human keratinocytes. Methods Enzymol 2014;535:371-87.
75. Kuo IH, Yoshida T, De Benedetto A, Beck LA. The cutaneous innate immune response in patients with atopic dermatitis. J Allergy Clin Immunol 2013;131:266-78.
76. Howell MD, Fairchild HR, Kim BE, Bin L, Boguniewicz M, Redzic JS, *et al*. Th2 cytokines act on S100/A11 to downregulate keratinocyte differentiation. J Invest Dermatol 2008;128:2248-58.
77. Altrichter S, Kriehuber E, Moser J, Valenta R, Kopp T, Stingl G. Serum IgE autoantibodies target keratinocytes in patients with atopic dermatitis. J Invest Dermatol 2008;128:2232-9.
78. Novak N, Bieber T, Leung DY. Immune mechanisms leading to atopic dermatitis. J Allergy Clin Immunol 2003;112:S128-39.
79. Noh M, Yeo H, Ko J, Kim HK, Lee CH. MAP17 is associated with the T-helper cell cytokine-induced down-regulation of filaggrin transcription in human keratinocytes. Exp Dermatol 2010;19:355-62.
80. Hatano Y, Adachi Y, Elias PM, Crumrine D, Sakai T, Kurahashi R, *et al*. The Th2 cytokine, interleukin-4, abrogates the cohesion of normal stratum corneum in mice: implications for pathogenesis of atopic dermatitis. Exp Dermatol 2013;22:30-5.
81. Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Méchin MC, *et al*. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. J Allergy Clin Immunol 2013;131:1094-102.
82. Deleuran M, Hvid M, Kemp K, Christensen GB, Deleuran B, Vestergaard C. IL-25 induces both inflammation and skin barrier dysfunction in atopic dermatitis. Chem Immunol Allergy 2012;96:45-9.
83. Gutowska-Owsiak D, Schaupp AL, Salimi M, Taylor S, Ogg GS. Interleukin-22 downregulates filaggrin expression and affects expression of profilaggrin processing enzymes. Br J Dermatol 2011;165:492-8.
84. Gutowska-Owsiak D, Schaupp AL, Salimi M, Selvakumar TA, McPherson T, Taylor S, *et al*. IL-17 downregulates filaggrin and affects keratinocyte expression of genes associated with cellular adhesion. Exp Dermatol 2012;21:104-10.
85. Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T. A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 2005;124:198-203.
86. Sajić D, Asiniwasis R, Skotnicki-Grant S. A look at epidermal barrier function in atopic dermatitis: physiologic lipid replacement and the role of ceramides. Skin Therapy Lett 2012;17:6-9.
87. Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ, *et al*. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. J Lipid Res 2012;53:2755-66.
88. Masukawa Y, Narita H, Sato H, Naoe A, Kondo N, Sugai Y, *et al*. Comprehensive quantification of ceramide species in human stratum corneum. J Lipid Res 2009;50:1708-19.
89. van Smeden J, Hoppel L, van der Heijden R, Hankemeier T, Vreeken RJ, Bouwstra JA. LC/MS analysis of stratum corneum lipids: ceramide profiling and discovery. J Lipid Res 2011:52:1211-21.
90. Bouwstra JA, Ponec M. The skin barrier in healthy and diseased state. Biochim Biophys Acta 2006;1758:2080-95.
91. Bouwstra JA, Gooris GS, van der Spek JA, Bras W. Structural investigations of human stratum corneum by small-angle X-ray scattering. J Invest Dermatol 1991;97:1005-12.
92. Ongpipattanakul B, Francoeur ML, Potts RO. Polymorphism in stratum corneum lipids. Biochim Biophys Acta 1994;1190:115-22.
93. Pilgram GS, Vissers DC, van der Meulen H, Pavel S, Lavrijsen SP, Bouwstra JA, *et al*. Aberrant lipid organization in stratum corneum of patients with atopic dermatitis and lamellar ichthyosis. J Invest Dermatol 2001;117:710-7.

94. Bleck O, Abeck D, Ring J, Hoppe U, Vietzke JP, Wolber R, *et al*. Two ceramide subfractions detectable in Cer(AS) position by HPTLC in skin surface lipids of non-lesional skin of atopic eczema. J Invest Dermatol 1999;113:894-900.

95. Farwanah H, Raith K, Neubert RH, Wohlrab J. Ceramide profiles of the uninvolved skin in atopic dermatitis and psoriasis are comparable to those of healthy skin. Arch Dermatol Res 2005;296:514-21.

96. Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A. Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? J Invest Dermatol 1991;96:523-6.

97. Ishikawa J, Narita H, Kondo N, Hotta M, Takagi Y, Masukawa Y, *et al*. Changes in the ceramide profile of atopic dermatitis patients. J Invest Dermatol 2010;130:2511-4.

98. Park YH, Jang WH, Seo JA, Park M, Lee TR, Park YH, *et al*. Decrease of ceramides with very long-chain fatty acids and downregulation of elongases in a murine atopic dermatitis model. J Invest Dermatol 2012;132:476-9.

99. Hachem JP, Man MQ, Crumrine D, Uchida Y, Brown BE, Rogiers V, *et al*. Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. J Invest Dermatol 2005;125:510-20.

100. Nakagawa N, Sakai S, Matsumoto M, Yamada K, Nagano M, Yuki T, *et al*. Relationship between NMF (lactate and potassium) content and the physical properties of the stratum corneum in healthy subjects. J Invest Dermatol 2004;122:755-63.

101. Schmid-Wendtner MH, Korting HC. The pH of the skin surface and its impact on the barrier function. Skin Pharmacol Physiol 2006;19:296-302.

102. Groen D, Poole DS, Gooris GS, Bouwstra JA. Is an orthorhombic lateral packing and a proper lamellar organization important for the skin barrier function? Biochim Biophys 2011;1808:1529-1737.

103. Gruber R, Elias PM, Crumrine D, Lin TK, Brandner JM, Hachem JP, *et al*. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. Am J Pathol 2011;178:2252-63.

104. Jungersted JM, Scheer H, Mempel M, Baurecht H, Cifuentes L, Høgh JK, *et al*. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. Allergy 2010;65:911-8.

105. Khnykin D, Rønnevig J, Johnsson M, Sitek JC, Blaas HG, Hausser I, *et al*. Ichthyosis prematurity syndrome: clinical evaluation of 17 families with a rare disorder of lipid metabolism. J Am Acad Dermatol 2012;66:606-16.

106. Sasaki T, Shiohama A, Kubo A, Kawasaki H, Ishida-Yamamoto A, Yamada T, *et al*. A homozygous nonsense mutation in the gene for Tmem79, a component for the lamellar granule secretory system, produces spontaneous eczema in an experimental model of atopic dermatitis. J Allergy Clin Immunol 2013;132:1111-20.

107. Perusquía-Ortiz AM, Oji V, Sauerland MC, Tarinski T, Zaraeva I, Seller N, *et al*. Complete filaggrin deficiency in ichthyosis vulgaris is associated with only moderate changes in epidermal permeability barrier function profile. J Eur Acad Dermatol Venereol 2013;27:1552-8.

108. Hachem JP, Wagberg F, Schmuth M, Crumrine D, Lissens W, Jayakumar A, *et al*. Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. J Invest Dermatol 2006;126:1609-21.

109. Nylander-Lundqvist E, Egelrud T. Formation of active IL-1 beta from pro-IL-1 beta catalyzed by stratum corneum chymotryptic enzyme in vitro. Acta Derm Venereol 1997;77:203-6.

110. Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C, *et al*. Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med 2009;206:1135-47.

111. Takai T. TSLP expression: cellular sources, triggers, and regulatory mechanisms. Allergol Int 2012;61:3-17.

112. Landheer J, Giovannone B, Mattson JD, Tjabringa S, Bruijnzeel-Koomen CA, McClanahan T, *et al*. Epicutaneous application of house dust mite induces thymic stromal lymphopoietin in nonlesional skin of patients with atopic dermatitis. J Allergy Clin Immunol 2013;132:1252-4.

113. Sano Y, Masuda K, Tamagawa-Mineoka R, Matsunaka H, Murakami Y, Yamashita R, *et al*. Thymic stromal lymphopoietin expression is increased in the horny layer of patients with atopic dermatitis. Clin Exp Immunol 2013;171:330-7.

114. Wilson SR, Thé L, Batia LM, Beattie K, Katibah GE, McClain SP, *et al*. The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. Cell 2013;155:285-95.

115. Kawasaki H, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, *et al*. Altered stratum corneum barrier and enhanced percutaneous immune respon-

ses in filaggrin-null mice. J Allergy Clin Immunol 2012;129:1538-46.

116. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, *et al*. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet 2009;41:602-8.

117. Jeong SK, Kim HJ, Youm JK, Ahn SK, Choi EH, Sohn MH, *et al*. Mite and cockroach allergens activate protease-activated receptor 2 and delay epidermal permeability barrier recovery. J Invest Dermatol 2008;128:1930-39.

118. Nylander-Lundqvist E, Bäck O, Egelrud T. IL-1 beta activation in human epidermis. J Immunol 1996;157:1699-704.

119. Lee KH, Cho KA, Kim JY, Kim JY, Baek JH, Woo SY, *et al*. Filaggrin knockdown and Toll-like receptor 3 (TLR3) stimulation enhanced the production of thymic stromal lymphopoietin (TSLP) from epidermal layers. Exp Dermatol 2011;20:149-51.

120. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. Nat Immunol 2010;11:289-93.

121. 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;339:b2433.

122. Elias PM.The skin barrier as an innate immune element. Semin Immunopathol 2007;29:3-14.

123. Lin YT, Wang CT, Chiang BL. Role of bacterial pathogens in atopic dermatitis. Clinic Rev Allerg Immunol 2007;33:167-77.

124. Williams RE, Gibson AG, Aitchison TC, Lever R, Mackie RM. Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis. Br J Dermatol 1990;123:493-501.

125. Aly R, Maibach HI, Shinefield HR. Microbial flora of atopic dermatitis. Arch Dermatol 1977;113:780-2.

126. Matsui K, Nishikawa A, Suto H, Tsuboi R, Ogawa H. Comparative study of Staphylococcus aureus isolated from lesional and non-lesional skin of atopic dermatitis patients. Microbiol Immunol 2000;44:945-7.

127. Aberg KM, Man MQ, Gallo RL, Ganz T, Crumrine D, Brown BE, *et al*. Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers. J Invest Dermatol 2008;128:917-25.

128. Baker BS. The role of microorganisms in atopic dermatitis. Clin Exp Immunol 2006;144:1-9.

129. Akiyama H, Toi Y, Kanzaki H, Tada J, Arata J. Prevalence of producers of enterotoxins and toxic shock syndrome toxin-1 among Staphylococcus aureus strains isolated from atopic dermatitis lesions. Arch Dermatol Res 1996;288:418-20.

130. Miller SJ, Aly R, Shinefeld HR, Elias PM. In vitro and in vivo antistaphylococcal activity of human stratum corneum lipids. Arch Dermatol 1988;124:209-15.

131. Bibel DJ, Aly R, Shinefield HR. Antimicrobial activity of sphingosines. J Invest Dermatol 1992;98:269-73.

132. Clarke SR, Mohamed R, Bian L, Routh AF, Kokai-Kun JF, Mond JJ, *et al*. The Staphylococcus aureus surface protein IsdA mediates resistance to innate defenses of human skin. Cell Host Microbe 2007;1:199-212.

133. Cho SH, Strickland I, Boguniewicz M, Leung DY. Fibronectin and fibrinogen contribute to the enhanced binding of Staphylococcus aureus to atopic skin. J Allergy Clin Immunol 2001;108:269-74.

134. Cho SH, Strickland I, Tomkinson A, Fehringer AP, Gelfand EW, Leung DY. Preferential binding of Staphylococcus aureus to skin sites of Th2-mediated inflammation in a murine model. J Invest Dermatol 2001;116:658-63.

135. Foster TJ, Höök M. Surface protein adhesins of Staphylococcus aureus. Trends Microbiol 1998;6:484-8.

136. Postlethwaite AE, Holness MA, Katai H, Raghow R. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. J Clin Invest 1992;90:1479-85.

137. Sator PG, Schmidt JB, Hönigsmann H. Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis. J Am Acad Dermatol 2003;48:352-8.

138. Kita K, Sueyoshi N, Okino N, Inagaki M, Ishida H, Kiso M, *et al*. Activation of bacterial ceramidase by anionic glycerophospholipids: possible involvement in ceramide hydrolysis on atopic skin by Pseudomonas ceramidase. Biochem J 2002;362:619-26.

139. Arikawa J, Ishibashi M, Kawashima M, Takagi Y, Ichikawa Y, Imokawa G. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by Staphylococcus aureus. J Invest Dermatol 2002;119:433-9.

140. Brauweiler AM, Bin L, Kim BE, Oyoshi MK, Geha RS, Goleva E, *et al*. Filaggrin-dependent secretion of sphingomyelinase protects against staphylococcal α-toxin-induced keratinocyte death. J Allergy Clin Immunol 2013;131:421-7.

141. Braff MH, Di Nardo A, Gallo RL. Keratinocytes store the antimicrobial peptide cathelicidin in lamellar bodies. J Invest Dermatol 2005;124:394-400.

142. Oren A, Ganz T, Liu L, Meerloo T. In human epidermis, beta-defensin 2 is packaged in lamellar bodies. Exp Mol Pathol 2003;74:180-2.

143. Aberg KM, Radek KA, Choi EH, Kim DK, Demerjian M, Hupe M, *et al*. Psychological stress downregulates epidermal antimicrobial peptide expression and increases severity of cutaneous infections in mice. J Clin Invest 2007;117:3339-49.

144. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, *et al*. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 2001;414:454-7.

145. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, *et al*. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Eng J Med 2002;347:1151-60.

146. Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, *et al*. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. J Immunol 2003;171:3262-9.

147. Zaiou M, Nizet V, Gallo RL. Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. J Invest Dermatol 2003;120:810-6.

148. Lomholt H, Andersen KE, Kilian M. Staphylococcus aureus clonal dynamics and virulence factors in children with atopic dermatitis. J Invest Dermatol 2005;125:977-82.

149. Leung DY, Hauk P, Strickland I, Travers JB, Norris DA. The role of superantigens in human diseases: therapeutic implications for the treatment of skin diseases. Br J Dermatol 1998;139:17-29.

150. Hauk PJ, Hamid QA, Chrousos GP, Leung DY. Induction of corticosteroid insensitivity in human PBMCs by microbial superantigens. J Allergy Clin Immunol 2000;105:782-7.

151. Miajlovic H, Fallon PG, Irvine AD, Foster TJ. Effect of filaggrin breakdown products on growth of and protein expression by Staphylococcus aureus. J Allergy Clin Immunol 2010;126:1184-90.

152. Cai SC, Chen H, Koh WP, Common JE, van Bever HP, McLean WH, *et al*. Filaggrin mutations are associated with recurrent skin infection in Singaporean Chinese patients with atopic dermatitis. Br J Dermatol 2012;166:200-3.

153. Wollenberg A. Eczema herpeticum. Chem Immunol Allergy 2012;96:89-95.

154. Wollenberg A, Wetzel S, Burgdorf WH, Haas J. Viral infections in atopic dermatitis: pathogenic aspects and clinical management. J Allergy Clin Immunol 2003;112:667-74.

155. Yoon M, Spear PG. Disruption of adherens junctions liberates nectin-1 to serve as receptor for herpes simplex virus and pseudorabies virus entry. J Virol 2002;76:7203-8.

156. Howell MD, Wollenberg A, Gallo RL, Flaig M, Streib JE, Wong C, *et al*. Cathelicidin deficiency predisposes to eczema herpeticum. J Allergy Clin Immunol 2006;117:836-41.

157. Wollenberg A, Wagner M, Günther S, Towarowski A, Tuma E, Moderer M. Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. J Invest Dermatol 2002:119:1096-102.

158. Leung DY, Gao PS, Grigoryev DN, Rafaels NM, Streib JE, Howell MD, *et al*. Human atopic dermatitis complicated by eczema herpeticum is associated with abnormalities in IFN-γ response. J Allergy Clin Immunol 2011;127:965-73.

159. Hinz T, Zaccaro D, Byron M, Brendes K, Krieg T, Novak N, *et al*. Atopic dermo-respiratory syndrome is a correlate of eczema herpeticum. Allergy 2011;66:925-33.

160. Bin L, Kim BE, Brauweiler A, Goleva E, Streib J, Ji Y, *et al*. Staphylococcus aureus α-toxin modulates skin host response to viral infection. J Allergy Clin Immunol 2012;130:683-91.e2.

161. Bin L, Edwards MG, Heiser R, Streib JE, Richers B, Hall CF, *et al*. Identification of novel gene signatures in patients with atopic dermatitis complicated by eczema herpeticum. J Allergy Clin Immunol 2014;134:848-55.