Published in final edited form as:
J Allergy Clin Immunol. 2008 June ; 121(6): 1337–1343. doi:10.1016/j.jaci.2008.01.022.

Basis for the barrier abnormality in atopic dermatitis: Outside-inside-outside pathogenic mechanisms

Peter M. Elias, MD[a,b], **Yutaka Hatano, MD, PhD**[a,b], and **Mary L. Williams, MD**[b,c]

[a]Dermatology Service, Veterans Affairs Medical Center, University of California, San Francisco, Calif

[b]Department of Dermatology, University of California, San Francisco, Calif

[c]Department of Pediatrics, University of California, San Francisco, Calif

Abstract

Until quite recently, the pathogenesis of atopic dermatitis (AD) has been attributed to primary abnormalities of the immune system. Intensive study revealed the key roles played by T_H1/T_H2 cell dysregulation, IgE production, mast cell hyperactivity, and dendritic cell signaling in the evolution of the chronic, pruritic, inflammatory dermatosis that characterizes AD. Accordingly, current therapy has been largely directed toward ameliorating T_H2-mediated inflammation and pruritus. In this review we will assess emerging evidence that inflammation in AD results from inherited and acquired insults to the barrier and the therapeutic implications of this paradigm.

Keywords

Antimicrobial peptides; atopic dermatitis; barrier function; barrier repair; cytokines; filaggrin; pH; psychologic stress; Staphylococcus aureus; serine proteases; T_H2 cells

Until recently, atopic dermatitis (AD) has been viewed largely as a disease of immunologic etiology.[1–5] Yet, the epidermis generates a set of protective/defensive functions (Table I) mediated by its unique differentiation end product, the stratum corneum (SC).[6,7] These functions include the permeability barrier, which retards transcutaneous evaporative water loss, allowing survival in a potentially desiccating external environment, and an antimicrobial barrier, which simultaneously encourages colonization by nonpathogenic ‘‘normal’’ flora while resisting growth of microbial pathogens.[8] Although both a defective epidermal permeability[9–13] and a propensity to secondary infection[14,15] are well-recognized features of AD, these abnormalities have been widely assumed to reflect downstream consequences of a primary immunologic abnormality (the historical inside-outside view of AD pathogenesis). We and others have long proposed that the permeability barrier abnormality inADis not merely an epiphenomenon but rather the ‘‘driver’’ of disease activity (ie, the reverse outside-inside view of disease pathogenesis)[16–19] for the following reasons: (1) the extent of the permeability barrier abnormality parallels the severity of the disease phenotype in AD[9,10,12]; (2) both clinically uninvolved skin sites and skin cleared of inflammation for as long as 5 years continue to display significant barrier abnormalities [10,13]; (3) emollient therapy comprises effective ancillary therapy [20]; and most importantly, (4) specific replacement therapy, which targets the

Reprint requests: Peter M. Elias, MD, Dermatology Service (190), VA Medical Center, 4150 Clement St, San Francisco, CA 94121. E-mail: E-mail: eliasp@derm.ucsf.edu. 0091-6749.

Disclosure of potential conflict of interest: P. M. Elias has served as a member for Ceragenix and has served as an expert witness in immunomodification and systemic retinoid litigation. M. L. Williams’ husband has served as a member of Ceragenix. Y. Hatano has declared that he has no conflict of interest.

prominent lipid abnormalities that account for the barrier abnormality in AD (see below), corrects both the permeability barrier abnormality and comprises effective anti-inflammatory therapy for AD (see the Therapeutic implications section below).

BROAD BARRIER FAILURE IN AD

Like permeability barrier dysfunction, the antimicrobial barrier is also compromised in patients with AD. Colonization by *Staphylococcus aureus* is a common feature of AD,[21] and although colonization is highest on lesional skin, colony counts often are high on the clinically normal skin of patients with AD.[14,15] Moreover, overt secondary infections, manifesting commonly as impetiginization, widespread folliculitis, or, less frequently, cutaneous abscesses or cellulitis, are well-recognized complications in the management of AD. Furthermore, colonization by superantigen-producing *S aureus* strains further exacerbates disease in patients with severe AD through generalized augmentation of IgE production, as well as through development of specific IgE directed toward staphylococcal exotoxins (see the "Impaired antimicrobial defense further compromises barrier function in AD" section below).[19] In addition, patients with AD are also susceptible to widespread cutaneous viral infections, including molluscum contagiosum, herpes simplex (Kaposi's varicelliform eruption), and life-threatening vaccinia.[22] Widespread dermatophytosis (tinea corporis) and *Malassezia* species infections also occur in AD, and the latter, such as *S aureus*, can stimulate specific IgE production.[22,23] Taken together, these observations point to loss of a competent antimicrobial barrier in AD. Although failure of both permeability and antimicrobial function is well recognized in patients with AD, only recently has it become clear that these 2 functions are both coregulated and interdependent.[24] Thus failure of the permeability barrier in itself favors secondary infection, and conversely, pathogen colonization/infection further aggravates the permeability barrier abnormality.

Finally, several other critical defensive functions of the SC are also compromised in patients with AD, including (1) SC integrity (cohesion), as reflected by excess scale (abnormal desquamation), and (2) diminished SC hydration, as reflected by lifelong cutaneous xerosis in these patients, even after overt inflammation recedes (Table I).[9,10,13] Like the defective permeability and antimicrobial barriers, SC hydration decreases in both the lesional and nonlesional skin of patients with AD, with its severity paralleling disease activity.[9,12] Decreased SC hydration is not merely of cosmetic concern because it alone suffices to stimulate epidermal hyperplasia and early evidence of inflammation, such as mast cell degranulation, even in normal skin.[25] Whether additional defensive functions of the SC, such as antioxidant or UV defense, also fail in patients with AD remain unknown. Nevertheless, AD can be viewed as a disease of broad barrier failure.

BASIS FOR THE PERMEABILITY BARRIER IN NORMAL SKIN

The permeability barrier resides in the SC, a multilayered tissue composed of flattened anucleate corneocytes surrounded by multiple planer lamellae sheets enriched in ceramides, cholesterol, and free fatty acids (FFAs).[26] It is the localization of these highly hydrophobic lipids within the extracellular domains of the SC that inhibits the outward movement of water. These lipids are delivered to the SC as their precursors through secretion of a unique organelle, the epidermal lamellar body (LB).[26] As the SC forms, this organelle delivers not only lipid constituents (eg, cholesterol) and lipid precursors (eg, glucosylceramides and phospholipids) but also the enzymes (β-glucocerebrosidase, acidic sphingomyelinase, and secretory phospholipase A_2) required to generate ceramides and FFAs, which are required for their organization into mature membrane structures.[26] In parallel, LB-derived proteases and their inhibitors orchestrate the orderly digestion of corneodesmosomes, transient intercellular junctions that are progressively degraded, allowing corneocytes to shed invisibly at the skin

surface.[27,28] Finally, antimicrobial peptides also are delivered to the SC intercellular domains through secretion of LB contents.[29–31]

INHERITED BARRIER ABNORMALITIES IN ATOPIC DERMATITIS

Based on inherited abnormalities either in serine protease (SP)/antiprotease expression or filaggrin (FLG) production, the development of AD is now increasingly linked to primary defects in the structure and function of the SC. The most compelling case for the role of excess SP activity in the pathogenesis of AD comes from Netherton syndrome (NS), an autosomal recessive disorder caused by loss-of-function mutations in *SPINK5*, the gene encoding the SP inhibitor lymphoepithelial Kazal-type trypsin inhibitor. [32] NS is characterized by severe AD, mucosal atopy, and anaphylactic reactions to food antigens.[25,26] Residual lymphoepithelial Kazal-type trypsin inhibitor expression in NS correlates inversely with excess SP activity within the outer epidermis,[33] resulting in a severe permeability barrier defect and dramatic thinning of the SC because of unrestricted, SP-dependent degradation of lipid-processing enzymes and corneodesmosome-constituent proteins, respectively.[33,34] Pertinently, several European, American, and Japanese case-control studies of patients with AD or mucosal atopy have found an increased frequency of single nucleotide polymorphisms (Glu420Lys) in *SPINK5*.[32] Conversely, a British case-control study described putative gain-of-function polymorphisms (AACCAACC vs AACC) in the 3′ region of *KLK7*,which encodes the SP SC chymotryptic enzyme or *KLK7*.[35] Moreover, transgenic mice forced to express human *KLK7* display a severe AD-like dermatosis.[36] Yet the incidence of both these polymorphisms is quite high in unaffected healthy patients,[37–39] and it is not yet known whether either of these single nucleotide polymorphisms alters expression of its respective protein product or products. Nevertheless, in experimental animals a net increase in SP activity, achieved by a variety of means, has been shown to compromise barrier function through accelerated degradation of both corneodesmosomes (accounting for flawed SC integrity) and degradation of extracellular lipid-processing enzymes (ie, β-glucocerebrosidase and acidic sphingomyelinase; Fig 1).[40] SP-mediated degradation of the extracellular hydrolytic enzymes would, in turn, result in a failure to generate ceramides, a characteristic lipid abnormality in AD.[41,42]

Increased SP activity likely provokes the barrier abnormality through a second and unrelated mechanism by signaling of the plasminogen activator type 2 receptor, which in turn down-regulates LB secretion,[43] entombing these organelles in nascent corneocytes.[44] Failure of LB secretion accounts, in turn, for another characteristic abnormality in AD, a global decrease in SC lipids,[11,45] which correlates with the observed decrease in extracellular lamellar bilayers[12] in patients with AD (Fig 1). Thus increased SP activity alone induces abnormalities that parallel those in AD, providing a mechanistic basis for the global reduction in extracellular lipids and further decrease in ceramide levels that occur in patients with AD.

The strongest evidence for a primary structural abnormality of SC underlying the pathogenesis of AD derives from the recent link between loss-of-function mutations in the gene encoding FLG and AD.[46–51] Up to 50% of European kindreds with AD reveal single- or double-allele or compound mutations in *FLG* on chromosome 1q21. Although 15 different mutations have been reported, the 2 most common (R501X and 2282del4) account for the majority of cases, [52] and because of their proximal location on the *FLG* gene, they also predict more severe loss of function.[53–55] Yet although the logic for the link between excess SP activity and the barrier abnormality in AD seems clear, how loss of FLG (an intracellular protein) provokes a permeability barrier abnormality (almost always an extracellular defect) is not known. Loss of this quantitatively important protein could alter corneocyte shape (eg, flattening) sufficiently to disrupt the organization of the extracellular lamellar bilayers. Alternatively, or in addition, FLG is generated during cornification as its precursor protein, profilaggrin, which is then

proteolytically processed into FLG during the abrupt transition from the granular cell layer to corneocyte.[56] Whereas FLG initially aggregates keratin filaments into keratin fibrils, subsequently, it is itself proteolytically degraded into amino acids, which are further deiminated into polycarboxylic acids, such as pyrrolidine carboxylic acid and trans-urocanic acid.[57] These metabolites, in turn, act as osmolytes, drawing water into corneocytes, thereby accounting in large part for corneocyte hydration (Fig 2). Hence the most immediate result of FLG deficiency in patients with AD is decreased SC hydration, leading in turn to a steeper water gradient across the SC, which likely drives increased transcutaneous water loss. Thus decreased SC hydration, leading to increased water loss, is the first and most obvious cause of barrier dysfunction in AD. However, neither corneocyte flattening nor decreased SC hydration alone would suffice to enhance antigen penetration, which is best explained by another consequence of FLG deficiency (ie, decreased downstream production of acidic metabolites resulting from FLG proteolysis). Indeed, trans-urocanic acid, in particular, is a purported, endogenous acidifier of the SC.[58] Thus decreased generation of FLG products could result in an initial increase in the pH of SC in patients with AD sufficient to increase the activities of the multiple SPs in SC (Fig 1), which all exhibit neutral-to-alkaline pH optima.[28] Such a pH-induced increase in SP activity, if prolonged, could precipitate downstream structural and functional alterations that would converge with those that result from inherited abnormalities in SP/antiprotease expression (Fig 1).

One important downstream consequence of increased SP activity is generation of the primary cytokines IL-1α and IL-1β[59,60] from their 33-kd proforms, which are stored in large quantities in the cytosol of corneocytes (Fig 1). The putative pH-induced increase in SP activity would generate 17-kd active forms of these cytokines,[60] the first step in the cytokin e cascade that we propose is a primary contributor to inflammation in AD (Fig 3). Sustained antigen ingress through a defective barrier leading to a T_H2-dominant infiltrate is a second cause of inflammation in AD.[50] Accordingly, correction of the barrier abnormality alone should ameliorate both causes of inflammation in AD.

EXOGENOUS AND ENDOGENOUS STRESSORS FURTHER AGGRAVATE BARRIER FUNCTION IN AD

Acquired pH-dependent increases in SP activity could also contribute to AD pathogenesis. That *FLG* mutations alone do not suffice is shown in ichthyosis vulgaris, where the same single- or double-allele *FLG* mutations reduce FLG content, but inflammation (ie, AD) does not always occur.[61,62] Certain stressors could elicit disease by aggravating the barrier abnormality by provoking an incremental increase in the pH of the SC, leading to a further amplification of SP activity. Such a barrier-dependent increase in pH (and SP activity) likely accounts for the precipitation of AD after the use of neutral-to-alkaline soaps (Fig 1), a well-known exogenous stressor of clinical AD.[63]

Prolonged exposure to reduced environmental humidity, as occurs in radiant-heated homes in temperate climates during the winter, is also a well-known risk factor for AD. Under these conditions, transcutaneous water loss would accelerate across a defective SC, aggravating the underlying permeability barrier abnormality and amplifying cytokine signaling of inflammation. Because FLG proteolysis is regulated by changes in external humidity,[57] sustained reductions in environmental relative humidity could further deplete residual FLG in single-allele *FLG*-deficient patients. Finally, sustained psychologic stress (PS) aggravates permeability barrier function in human subjects,[64,65] and PS is both a well-known precipitant of AD and a cause of resistance to therapy. In the case of PS, however, the likely mechanism differs from either surfactant use or decreased environmental humidity. In experimental animals psychologic stress induces an increase in endogenous glucocorticoids (GCs), which in turn alter permeability barrier homeostasis, SC integrity, and epidermal antimicrobial

defense.[31,66,67] The putative mechanism for the negative effects of psychologic stress is GC-mediated inhibition of synthesis of the 3 key epidermal lipids that mediate barrier function (ie, ceramides, cholesterol, and FFAs).[68] Accordingly, a topical mixture of these 3 lipids largely normalizes all of these functions, even in the face of ongoing PS or GC therapy.[31,68]

OUTSIDE-INSIDE AND THEN BACK TO OUTSIDE PATHOGENIC MECHANISM IN AD

Despite accumulating evidence in support of a barrier-initiated pathogenesis of AD, recent studies suggest specific mechanisms whereby T_H2-generated cytokines could also further aggravate AD. Exogenous applications of the T_H2 cytokine IL-4 impede permeability barrier recovery after acute perturbations.[69] The basis for the negative effects of IL-4 could include (1) the observation that exogenous IL-4 also inhibits ceramide synthesis,[70] providing yet another mechanism accounting for decreased ceramide levels; (2) the observation that IL-4 also was shown recently to inhibit expression of keratinocyte differentiation-linked proteins, most notably FLG[71]; and (3) the observation that desmoglein 3 expression is also inhibited by exogenous IL-4.[72] Together, these observations provide acquired mechanisms that could further compromise barrier function in patients with AD.[71,72] Thus primary inherited barrier abnormalities in AD ultimately stimulate downstream paracrine mechanisms that could further compromise permeability barrier function, completing a potential outside-inside-outside pathogenic loop in AD (Fig 3).

IMPAIRED ANTIMICROBIAL DEFENSE FURTHER COMPROMISES BARRIER FUNCTION IN AD

In the prior sections, we discussed first how genetic and acquired factors can converge to provoke or amplify AD and second how inflammation can be attributed both to an epidermis-derived cytokine cascade, as well as to stimulation of a T_H2-dominant inflammatory infiltrate because of sustained antigen ingress. Increased colonization with *S aureus*[2,14,73] occurs as a result of the barrier abnormality (a structurally competent, lipid-replete, acidic SC itself comprises a formidable barrier to pathogen colonization[8]), and it can further aggravate barrier function in AD through several mechanisms (Fig 4). The antimicrobial barrier is intimately linked to the permeability barrier,[24] and as with water egress, pathogen ingress occurs through the extracellular domains. [74] Moreover, an impaired permeability barrier alone predisposes to pathogen colonization, not only because of the increase in surface pH[75] but also because levels of FFAs and the ceramide metabolite sphingosine, which exhibit potent antimicrobial activity,[74,76] are reduced in AD.[8] Surface proteins on *S aureus* can downregulate epidermal FFA production,[77] thereby aggravating both permeability and antimicrobial function in parallel, a strategy that could also facilitate microbial invasion. In addition, members of 2 key families of antimicrobial peptides, the human cathelicidin product LL-37 and human β-defensins 2 and 3, are downregulated in a T_H2-dependent fashion in AD (Fig 4).[73,78] Notably, both the human cathelicidin aminoterminal fragment cathelin[79] and human β-defensin 3[80,81] display robust activity against *S aureus*. LL-37 is required for normal epidermal permeability barrier function (notably, LL-37 is also important for the integrity of extracutaneous epithelia).[22] Thus it is likely that decreased LL-37 levels amplify the barrier defect in AD (Fig 4).

Over time, nontoxigenic strains of *S aureus* that colonize patients with AD can be replaced by enterotoxin-generating strains,[82] which in turn could aggravate AD through at least 3 mechanisms (Fig 4): (1) toxigenic strains are more likely to produce clinical infections than are nontoxigenic strains[82]; (2) some toxins stimulate pruritus[83] and production of specific IgE[15,84–86]; and (3) some toxins serve as “superantigens” that stimulate T- and B-cell proliferation, as well as immunoglobulin class-switching to allergen specific or

"superallergens" that stimulate IgE production.[15,87] Activated T cells produce IL-31, which also induces pruritus.[88] Finally, clinical infections, particularly folliculitis, are notoriously pruritic, even in nonatopic subjects, eliciting an itch-scratch vicious cycle that creates additional portals of entry for pathogens (Fig 4). It is self-evident that excoriations create further defects in the permeability barrier, representing yet another potentially important vicious cycle in AD pathogenesis (Fig 4).

THERAPEUTIC IMPLICATIONS

Together, the converging pathogenic features described above create a strong rationale for the deployment of specific strategies to restore barrier function in patients with AD. Based on the mechanisms described above, these approaches could range from a simple reduction in the pH of SC alone (hyperacidification), applications of SP inhibitors, topical plasminogen activator type 2 receptor antagonists, general moisturization measures, or specific lipid replacement therapy. Moisturizers are widely used in AD and, when used under nursing supervision, have been shown to reduce topical steroid use.[20] Of these approaches, the last is well into development as triple-lipid, ceramide-dominant, barrier repair therapy for AD, provided in an acidic formulation.* Two clinical studies support the efficacy of targeted, ceramidedominant lipid replacement therapy in AD. An open-label study demonstrated dramatic improvements in clinical activity, permeability barrier function, and SC integrity when an over-the-counter version of this technology (TriCeram; Osmotics Corp, Denver Colo) was substituted for standard moisturizers in children with severe recalcitrant AD.[12] More recently, a higher-strength, US Food and Drug Administration–approved prescription formulation (EpiCeram cream; Ceragenix Corp, Denver, Colo) demonstrated efficacy that was comparable with that of a midpotency steroid (fluticasone, Cutivate cream) in an investigator-blinded, multicenter clinical trial of pediatric patients with moderate-to-severe AD.[89] These studies, although still preliminary, suggest that pathogenesis-based therapy, directed at the lipid biochemical abnormality that is responsible for the barrier defect in AD, could comprise a new paradigm for the therapy of AD. Yet an important question remains: Will restoration of permeability barrier function simultaneously improve antimicrobial defense in patients with AD? Because recent studies have shown that these 2 key functions are both regulated in parallel and interdependent, [24] there is reason to be optimistic on this score as well.

Abbreviations used

AD, Atopic dermatitis; FFA, Free fatty acid; FLG, Filaggrin; GC, Glucocorticoid; LB, Lamellar body; NS, Netherton syndrome; SC, Stratum corneum; SP, Serine protease.

Acknowledgments

Supported by National Institutes of Health grant AR19098 and Department of Defense grant W81XWH-05-2-0094 and by the Medical Research Service, Department of Veterans Affairs.

We gratefully acknowledge the superb editorial assistance of Ms Joan Wakefield, including her preparation of the graphics.

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*Dr Elias is a coinventor of this University of California patented technology and is an officer of Ceragenix Corporation, the licensee of this technology.

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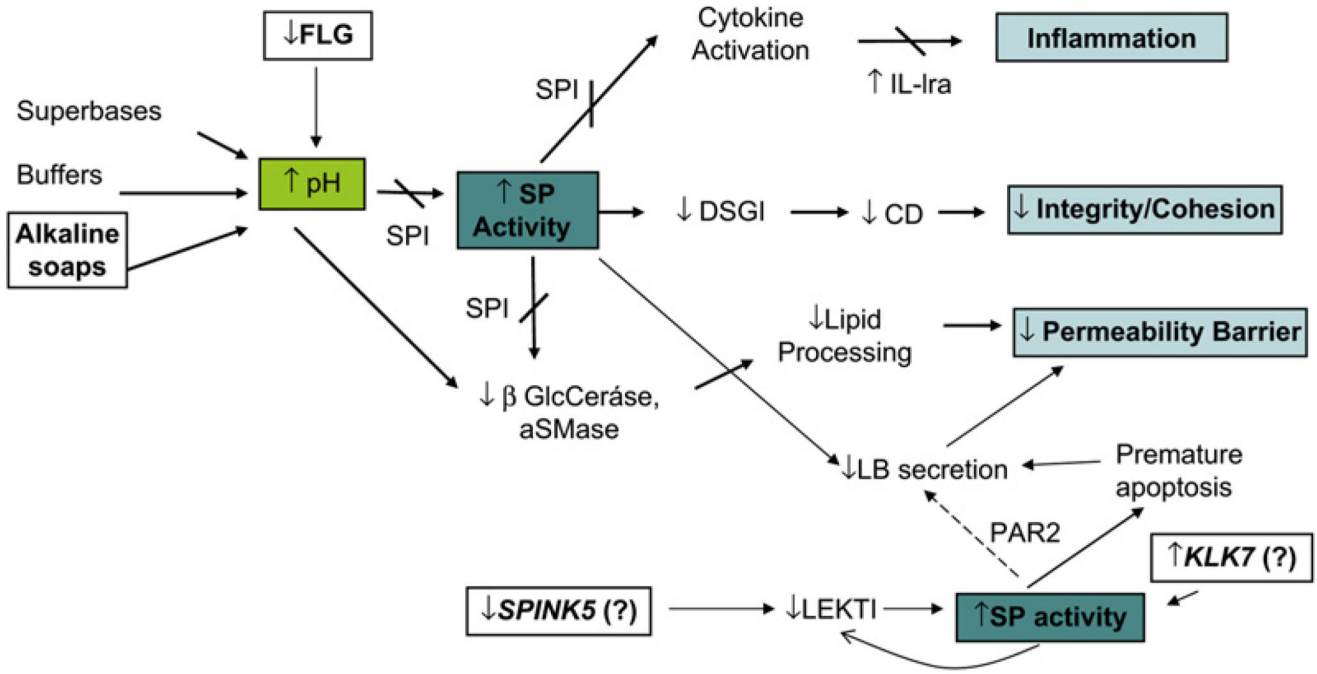

FIG 1.

Inherited and acquired activation of serine proteases converge to affect multiple SC functions but by divergent mechanisms. *SPI*, Serine protease inhibitor; *DSG1*, desmoglein 1; *CD*, corneodesmosome; *LEKTI*, lymphoepithelial Kazal-type trypsin inhibitor; *PAR2*, plasminogen activator type 2 receptor;*KLK7*, *kallikrein 7*.

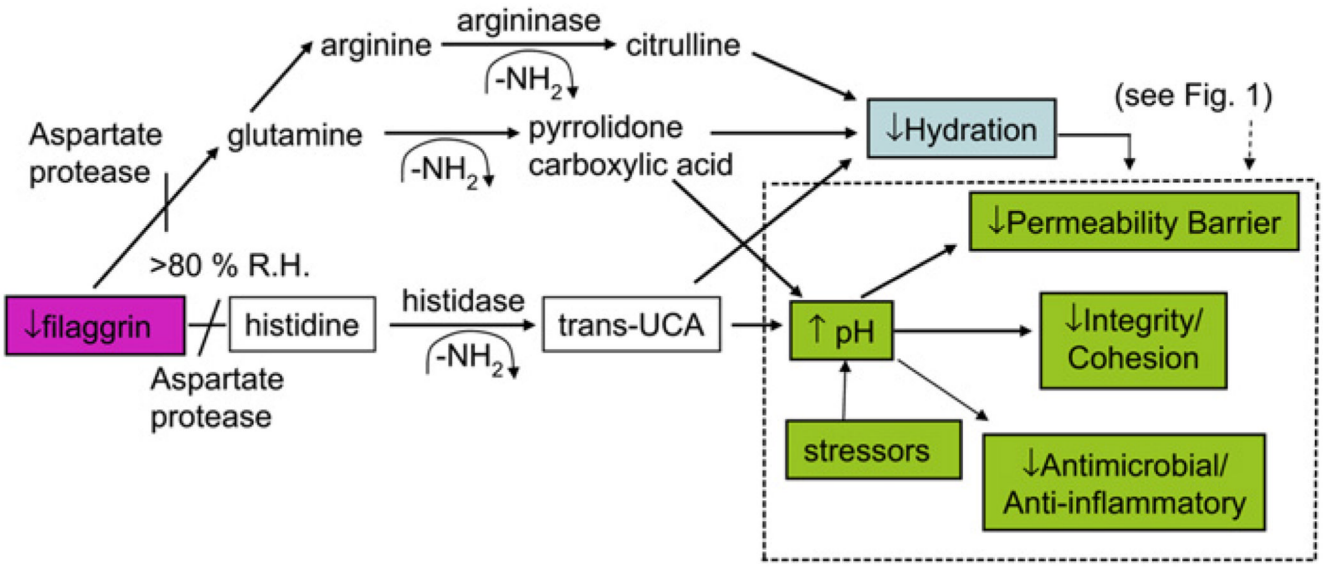

FIG 2.

FLG proteolytic pathway affects multiple SC functions: potential implications for pathogenesis of AD. *R.H.*, Relative humidity; *trans-UCA*, trans-urocanic acid.

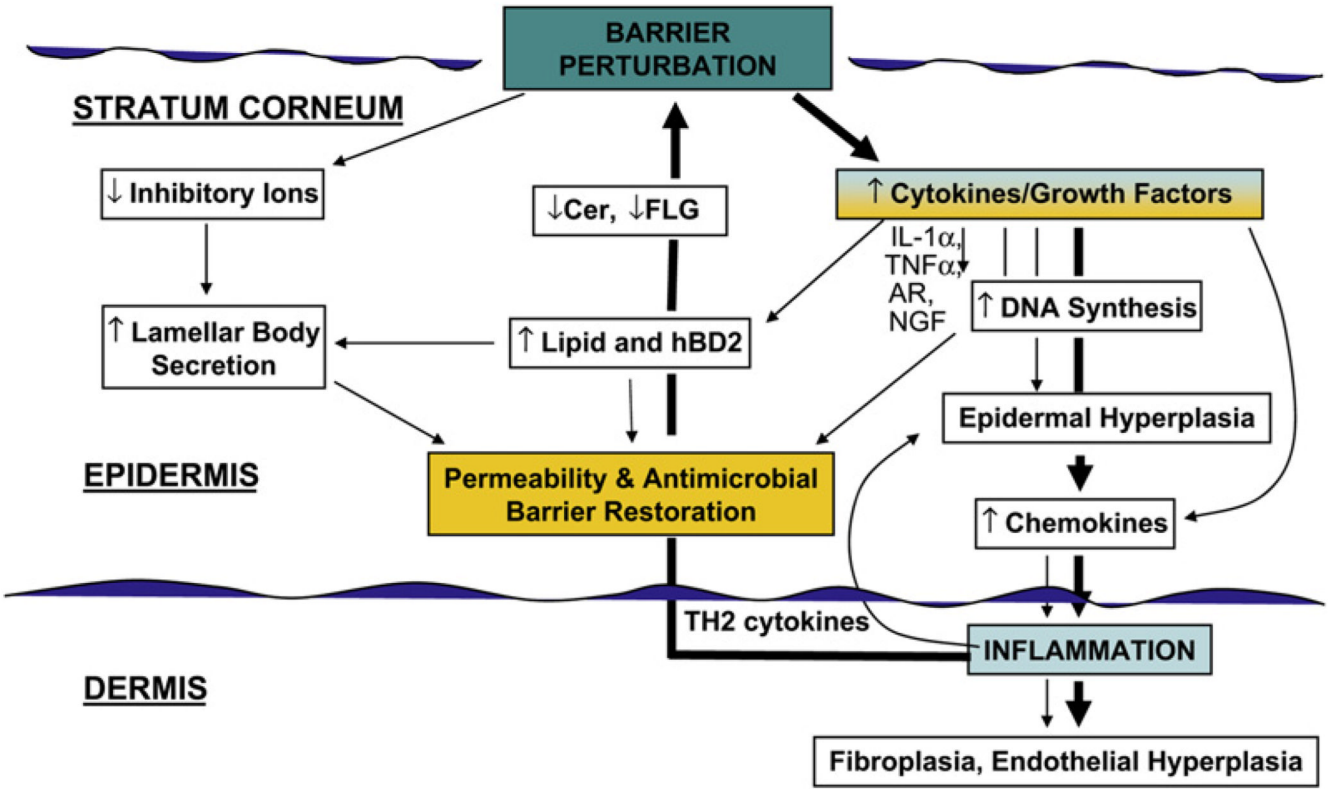

FIG 3.

Outside-inside initial provocation of AD eventually can lead to an outside-inside-outside vicious cycle. *hBD2*, Human β-defensin; *AR*, amphiregulin; *NGF*, nerve growth factor.

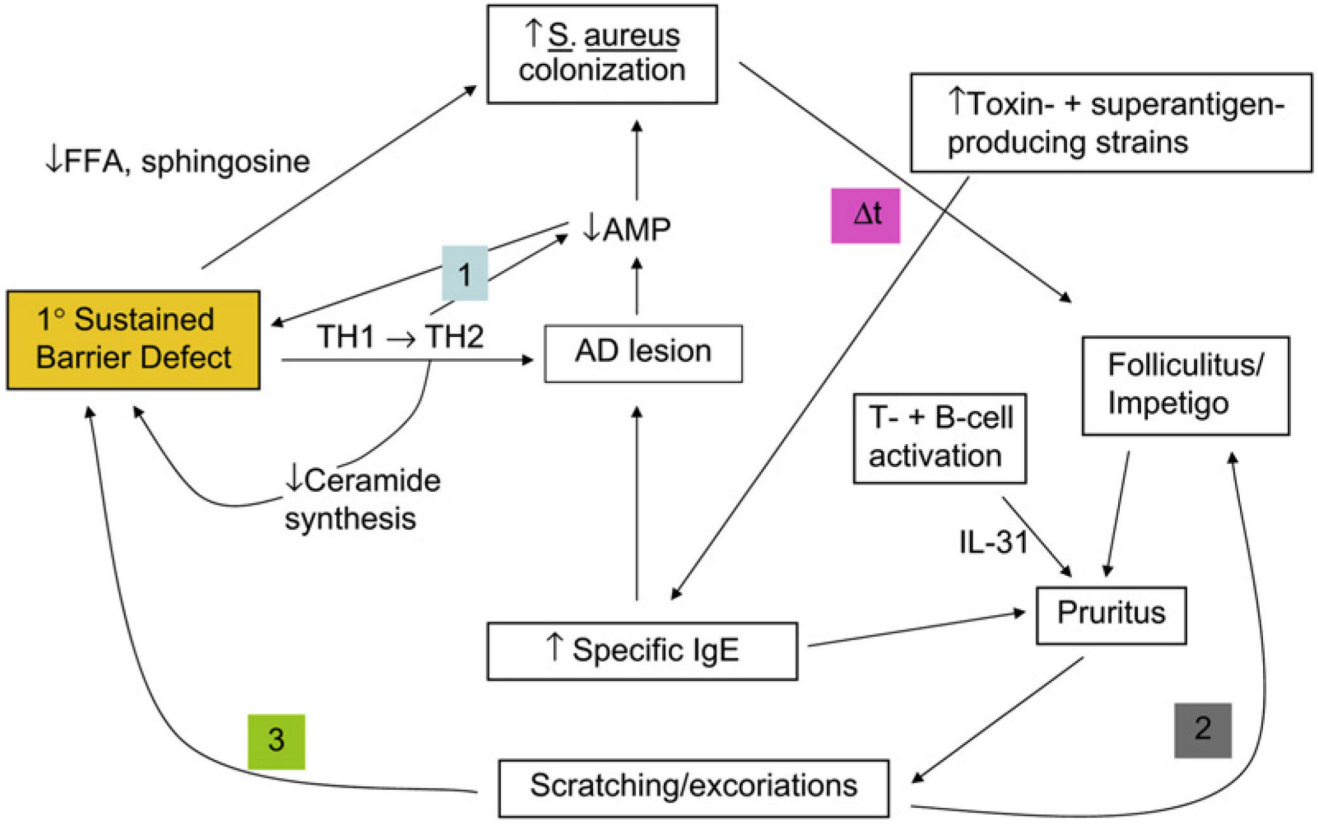

FIG 4.
Role of secondary infections in further aggravation of AD. *AMP*, Antimicrobial peptides; *FFA*, free fatty acids.

TABLE I

Multiple protective functions of mammalian SC

Function	Principal compartment	Structural basis	Chemical basis	Regulatory signals (rec
Permeability*†	Extracellular matrix	Lamellar bilayers	Ceramides, cholesterol, nonessential fatty acids in proper ratio	IL-1α Ca^{++}, pH, liposen serine proteases through TPRV1 and TPRV4
Antimicrobial*†	Extracellular matrix	Lamellar bilayers	Antimicrobial peptides, FFAs, Sph	1,25 $(OH)_2D_3$, IL-1α
Antioxidant†	Extracellular matrix	Lamellar bilayers	Cholesterol, FFAs, secreted vitamin E, redox gradient	?
Cohesion (integrity) → desquamation*†	Extracellular matrix	CD	Intercellular DSG1/DSC1 homodimers	pH, Ca^{++} (TPRV)
Mechanical/rheologic†	Corneocyte	Cornified envelope, keratin filaments	γ-Glutamyl isopeptide bonds	Ca^{++}, $CholSO_4$, liposens
Chemical (antigen exclusion)*†	Extracellular matrix	Extracellular lacunae	Hydrophilic products of CD	Same as for permeability
Psychosensory interface†	Extracellular matrix	Lamellar bilayers	Barrier lipids	GCs, heat (TPRV3)
Hydration†	Corneocyte	Cytosol	FLG proteolytic products, glycerol	Osmotic changes (TPRV TPRV4), aquaporin 3
UV light	Corneocyte	Cytosol	Trans-urocanic acid (histidase activity)	
Initiation of inflammation (1° cytokine activation)*†	Corneocyte	Cytosol	Proteolytic activation of pro-IL-1α/β	pH, serine protease activ

TPRV, Transient receptor potential vanilloid; *Sph*, sphingomyelin; *CD*, corneodesmosomes; *DSG1*, desmoglein 1; *DSC1*, desmocollin 1.

*Regulated by SC pH.

†Abnormal in AD.