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Pathophysiology of atopic dermatitis: Stratum corneum as a permeability barrier.

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Introduction

Atopic dermatitis is a familial inflammatory skin disease with a chronically relapsing course. The disease is characterized by lichenified pruritic eczematous lesions. Flexural site eczema and dry skin are among the other features. The pathogenesis remains unclear and represents a complex inter-relationship between genetic, immunological and environmental factors[1) 2) 3)]. In this review I will describe the pathophysiology of atopic dermatitis in terms of stratum corneum barrier function.

Histogenesis of Atopic Dermatitis

The histopathology of atopic dermatitis is similar to that of an eczematous tissue reaction. This is one of the prototypes of cell-mediated immunity called type Ⅳ allergic reaction. In contrast, other atopic disorders such as bronchial asthma, allergic rhinitis and allergic conjunctivitis are IgE-mediated immune response, called type Ⅰ allergic reaction.

Concomitant with other atopic disorders, serum IgE level is mostly increased in atopic dermatitis patients. The significance of the finding in terms of pathophysiology, however, remains to be determined. IgE is typically associated with allergic urticaria. Since scratching results in a lichenified eczematous change, the specific IgE-mediated subclinical urticaria might be a factor. One should note that atopic dermatitis is characterized by severe itching as well as lichenification. Based on this fact, the IgE-mediated itch-scratch-lichenification cycle is the basic pathomechanism of atopic dermatitis. This vicious cycle should be prevented by use of appropriate anti-pruritic agents or via inhibition of release of inflammatory chemical mediators.

Another possible mechanism in terms of IgE is the IgE-Fc receptor-mediated reaction associated with Langerhans cells[4) 5) 6)]. Langerhans cells are dendritic immune competent cells present in the epidermis. They are derived from bone-marrow and are involved in antigen presentation in a classical contact allergy mechanism. Recent evidence indicates high affinity IgE-Fc receptors on Langerhans cells that might be activated by specific antigens. Bruynzeel-Koomen[4)] showed specific IgE immunogold labeling on Langerhans cells. This Langerhans cell-bound IgE could be directly related to the eczematous tissue reaction. It is interesting to note that IgE-bearing Langerhans cells derived from atopic dermatitis skin are superior to IgE^--Langerhans cells in their capacity to present house dust antigen to sensitized T cells[6)]. This suggests that the specific IgE on Langerhans cells may play a role in the uptake and/or processing of allergens by these cells during the antigen presentation process.

Lesional skin of atopic dermatitis exhibits an increased number of dermal Langerhans cells[7)]; the epidermal Langerhans cells of atopic dermatitis possess hyperstimulatory antigen presenting activity with abnormal phenotypes[3)]. It has been reported that $CD4^+$ T cells repeatedly stimulated with activated Langerhans cells differentiate into T lymphocytes that produce high amounts of IL-4 and negligible IFN γ[8)]. IL-4-producing $CD4^+$ T lymphocytes are increased in atopic dermatitis lesional skin[9)]. Because IL-4 induces IgE synthesis and the expression of receptors for IgE on cells, a positive feedback loop of IgE-dependent process might be operative in atopic dermatitis[3)]. Conversely IFN γ counteracts the effects of IL-4[10)];beneficial effects of IFN γ in the treatment of atopic dermatitis have been described[11)].

Functional Aspects of Stratum Corneum

Recent studies have focused on the functional aspects of stratum corneum, the outermost layer of the epidermis

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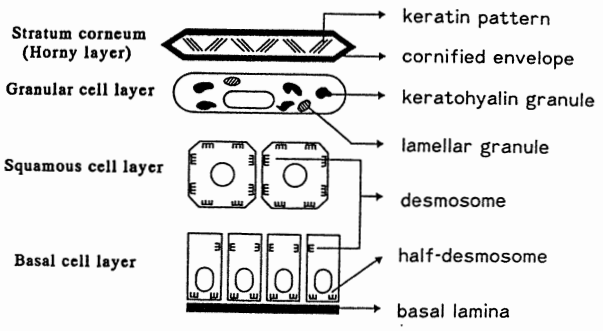


fig. 1 Schematic diagram of the epidermis.

Columnar basal cells go up and differentiate to be squamous cells, granular cells, and then to dead, flattened corneocytes. Although the epidermis is a compact tissue (see also Figure 2) representative cells of each cell layer are shown. Granular cells are characterized by keratohyalin granules. Lamellar granules appear at upper squamous cell layers and in granular cell layers. They contain stacks of membraneous disks, which extrude their lipid contents to the spaces between the uppermost layer of the viable epidermis and the corneocytes. Corneocytes are characterized by keratin pattern and cornified cell envelope that lines inner surface of corneocyte cell 'membrane'.

(Figure 1). This is a thin and flexible protective membrane, that consists of about 20 tightly stacked layers of dead epidermal cells, called corneocytes. Although the stratum corneum is only about 20 μm thick, it plays a crucial role by maintaining the barrier function that both protects the body from dessication and prevents invasion by exogenous toxic agents as well as contact allergens. Water-holding capacity is another important function of the stratum corneum.

As described below, the stratum corneum of atopic dermatitis patients is markedly deranged, which results in a defective barrier function. In other words, various water-soluble antigens would more easily permeate through the defective atopic stratum corneum. Water-holding capacity of atopic stratum corneum is also decreased to result in typical atopic dry skin.

Morphological Aspects of Corneocytes

Corneocyte morphology and its lipid composition are important determinants for both water-retention and permeability barrier function. It has been shown that the stratum corneum has a water permeability thousands of times lower than that of most of the other biomembranes[12]. This is related to its unique morphology that is analogous to highly impermeable barriers formed by the inclusion of flakes in a homogeneous matrix. The flattened hexagonal-shaped corneocytes correspond to the flakes and intercellular lipids correspond to the matrix. Note that the lipid membrane itself is relatively permeable to water. Because of the flattened hexagonal-shaped impermeable flakes, the stratum corneum is almost impermeable to water. The hexagonal shape is derived from a 14 sided tetrakaidecahedron structure of compactly-packed keratinocytes.

Flattened Corneocytes are Typically Hexagonal

The epidermis is a compact tissue. Columnar basal cells divide supplying new keratinocytes, go up and differentiate to become squamous cells, and then to dead, flattened corneocytes that form the stratum corneum. Corneocytes will then be desquamated or shed off. Thus the steady state of the epidermal architecture is maintained.

The squamous cell layer is located between the lowermost columnar basal cells and the uppermost flattened corneocytes. Despite that a spherical configuration is most stable for isolated keratinocytes, squamous cells are not spherical;they adopt a 14 sided tetrakaidecahedron structure to attain the compact epidermal architecture (Figure 2)[13) 14)]. Tetrakaidecahedron,

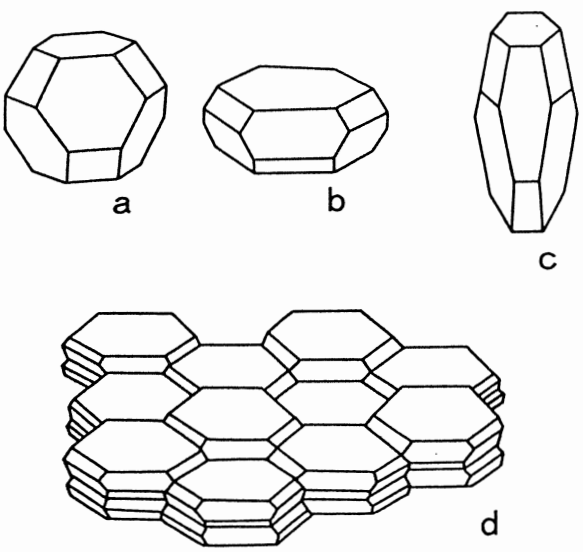


fig. 2 Tetrakaidecahedral nature of epidermal keratinocytes. Modified from Allen and Potten[14]

a. Line drawing of an orthic tetrakaidecahedron. This 14-sided figure consists of six equal opposite quadrilateral faces and eight equal and opposite hexagons. This corresponds to typical keratinocytes in squamous cell layer.

b. When compressed about a pair of hexagonal faces, the shape will become predominantly hexagonal. This corresponds to a typical corneocyte.

c. Elongated tetrakaidecahedron. Basal cells would be more close to a hexagonal column.

d. Flattened tetrakaidecahedra assembled showing stratum corneum.

when compressed and flattened, become hexagonal-shaped

fig. 3 Cussler's barrier membrane theory.

Cussler [15] defines the ratio of flux without impermeable blocks (Jo) to that with n layers of impermeable blocks (Jn) as:

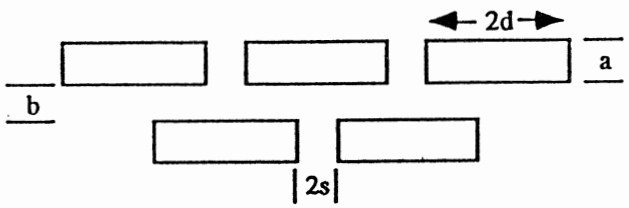
$$Jo/Jn = 1 + \sigma\alpha\phi + \alpha^2\frac{\phi^2}{1-\phi}$$

where α (flake aspect ratio)$= \frac{d}{a}$

ϕ (volume fraction flakes in membrane)$= \frac{b}{a+b}$

σ (pore aspect ratio)$= \frac{s}{a}$

a = thickness of the block
2d = width of the block
2s = lateral spacing between blocks
b = vertical spacing between blocks



Under the conditions where s << d

$$Jo/Jn = 1 + \alpha^2\frac{\phi^2}{1-\phi} \qquad \text{----- (2)}$$

For stratum corneum:

a=0.5 μm
b=0.1 μm
c=15 μm
d=0.05 μm

$$Jo/Jn = 1 + 30^2 \times \left(\frac{0.69}{1-0.83}\right) = 3{,}751.$$

Thus the permeability of the stratum corneum would be $\sim$ 4,000-fold greater than without corneocytes [12].

Note that the most influential factor in equation (2) is α (flake aspect ratio).

corneocytes of the stratum corneum. This is because of the tetrakaidecahedral nature of squamous cells underneath.

Why then should corneocytes be flattened? Why should corneocytes adopt such an unstable shape? Orthic tetrakaidecahedron is more stable in a compact architectural condition.

Because of the flattened corneocytes, the stratum corneum possesses permeability barrier function. Keratinocytes should be flattened or horizontally-extended to possess permeability barrier function or protect the body from dessication.

Barrier Membrance Theory

Cussler et al[15] presented a barrier membrane theory that defines how membranes which contain impermeable flakes can show permeabilities much lower than conventional membranes, and hence can serve as barriers for water, oxygen and other solutes (Figure 3).

According to the theory, a highly impermeable barrier can be formed by the inclusion of impermeable flakes in a homogeneous matrix. The incorporation of flakes into a homogeneous matrix can reduce the permeability by orders of magnitude compared to the pure phase.

Note that in this situation among the most influential factors for permeability barrier is factor α (flake aspect ratio), that is flake size divided by flake thickness (Figure 3). Any increase in this factor results in the increase of the barrier function. This means that flatter corneocytes are more efficacious for the permeability barrier function. Corneocytes should be flattened to attain permeability barrier function.

In atopic dermatitis the size of corneocytes is smaller than normal;this is observed even in non-eczematous atopic dry skin[16]. When we assume that the size is decreased with a concomitant increase in the thickness of corneocytes, the barrier function of atopic stratum corneum is considerably decreased. The decreased size is related to accelerated epidermal turnover in atopic dermatitis, because time is required for corneocytes to spread sufficiently. Psoriasis, another hyperproliferative skin disease with a markedly decreased turnover time, is also characterized by smaller corneocytes[17].

Intercellular Lipids: Matrix of the Stratum Corneum

Intercellular Lipids, the matrix of the stratum corneum, are another important determinant of permeability barrier function.

The stratum corneum contains about 14% by weight of lipid that is extractable with polar solvents. This lipid consists of ceramides(40－50%), free fatty acids(15－25%), cholesterol(20－25%), and cholesterol sulfate(5－10%)[18]. Phopholipids, a major constituent of living cell membrane, is virtually lacking. Cholesterol sulfate is assumed to be involved in interlamellar adhesion, with its hydrolysis resulting in a loosening of these attachments. Ceramide survives during desquamation process intact while cholesterol sulfate decreases markedly.

Ceramides, or N-acylsphingosines, are the major constituent of stratum corneum lipids. They are derived from lamellar granules, round or oval-shaped, approximately 100 x 300 nm-sized intracytoplasmic granules, that discharge their lipid contents to the intercellular spaces between the uppermost layer of the viable epidermis and the corneocytes (Figure 1). Ceramides can be separated into at least 6 distinguishable fractions (Figure 4)[18) 19]. Ceramide 1 is the least

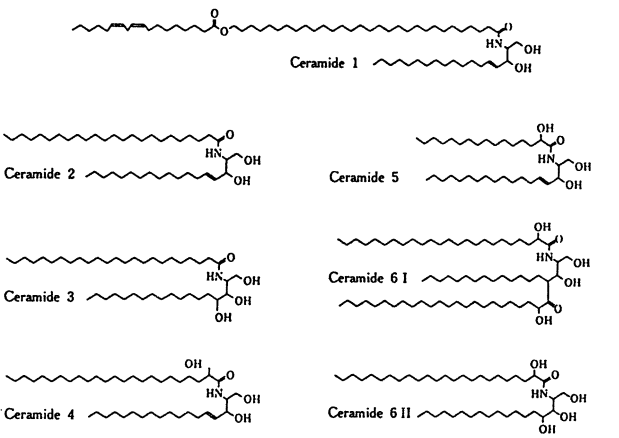


fig. 4 Representative structure of epidermal ceramides. Pig epidermal ceramides are shown according to Wertz and Downing [26].

polar or hydrophobic and ceramise 6 is the most polar or hydrophilic. The ceramide 1 is unique in its structure. It has a very long ω-acyl-oxy acyl chain with esterified linoleic acid at the ω-position[18]. Epidermosides, glucosyl-(ω-o-linoleoyl)-acylsphingosines[20], probably represent the precursor of the ceramide 1 in human.

Note that the carbon number of the acyl chain of ceramide 1 is 30－34, so long that it cannot fit into the conventional lipid biomembrane which is composed of phospholipid bilayers of about 16－18 carbon long. Furthermore, the presence of ω-hydroxy group at the end of the long acyl chain indicates its hydrophilic nature at this area. ω-Hydroxy ceramide with this very long chain was detected to be covalently attached to

the corneocyte protein envelopes[18].

Swartzendruber et al[19] proposed a molecular model of the intercellular lipid lamellae in mammalian stratum corneum (Figure 5). According to this model, deacylated ceramide 1 is

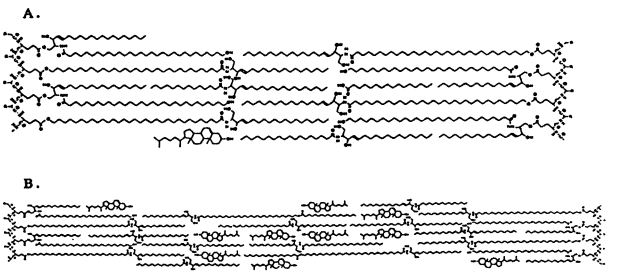


fig. 5 Proposed molecular arrangements of intercellular lipid lamellae.
A:Two apposed corneocyte lipid envelopes separated by monolayer lipid.
B:Two corneocyte lipid envelopes with lipid monolayers. This results in intercellular lamellae pattern. Modified from Swartzendruber et al[19].

bound to corneocyte membranous protein and is fixed or immobile. Note that the alternative positioning of the deacylated ceramide 1 will fit the positions of other ceramides as well as sterols. Although this configuration for double-chained lipid has not been established, this would certainly help to explain the remarkable cohesion between bilayers that characterize the stratum corneum intercellular lamellae. The least polar deacylated hydroxyceramide 1 is the most important determinant of barrier function because of its hydrophobic nature as well as its immobility. Corneocytes become "water-proof" due to the fixed hydroxyceramide that coats the corneocyte surface.

Hydrophilic ceramides 2－6 with relatively short, non-branched chains or with more hydroxylated polar heads are assumed to be associated with water holding function.

Ceramides are Decreased in Atopic Dermatitis Corneocytes.

In atopic dermatitis all ceramides 1－6 are decreased. Among the ceramide fractions, ceramide 1 is most significantly decreased[21,22]. The decrease is observed in both lesional and non-lesional skin. This results in the dry and more permeable stratum corneum of atopic dermatitis skin.

Antigens that permeate through the Defective Atopic Stratum Corneum

The stratum corneum of atopic dermatitis is characterized by smaller corneocytes and a decreased ceramide content. Both findings are consistent with the defective barrier function of the atopic stratum corneum. Various antigens would more easily permeate through the defective stratum corneum.

Dust mite [Dermatophagoides pteronyssinus and Dermatophagoides farinei] antigens are among the major antigens detected in atopic dermatitis patients. Thirty to forty percent of patients with atopic dermatitis show positive responses to patch testing with dust mite antigens, which mostly correlate with elevated levels of specific IgE to these antigens[23]. Imayama et al, however, proposed that combination of patch test and IgE for dust mite antigens differentiate atopic dermatitis into four groups with distinct clinical features[23].

Dust mites are ubiquitous in our environments. Enormous amounts of dust mite antigens are detected from carpets, Japanese mattresses, etc. Both proteins extracted from the whole body of mites and from feces have antigenicity. The antigens can be detected in about half of the cases of naturally occurring lesions of atopic dermatitis[24].

Because of the defective barrier function, atopic dermatitis patients may more easily be sensitized by environmental contact antigens including those of dust mites. If the atopic dermatitis is induced by the contact allergy mechanism related to the defective barrier function, the significance of the increased IgE becomes ambiguous. Increased IgE might simply be one of the concomitant features of atopic dermatitis patients and not necessarily involved in its pathophysiology. This is consistent with the finding that atopic dermatitis may be observed in agammaglobulinemia patients, who possess no IgE.

It must be mentioned, however, that the dust mite antigens are relatively large molecules with molecular weights of about 10,000－30,000[25,26]. This is far greater than those of the conventional contact allergens (haptens). They may pass through the scratched and eroded stratum corneum but not through the uninvolved atopic stratum corneum, if it is more permeable than the 'normal' stratum corneum. Although many of the atopic dermatitis patients show positive patch testing to mite antigents, adhesive tape stripping or somewhat abraded skin is usually required for a positive patch test[23,27]. IgE-dependent pruritus, because of the resultant scratching process, then might be significantly involved in the pathophysiology of atopic dermatitis. Since dust mite antigens are ubiquitous in our environments, the vicious cycle of re-challanging by the dust mite antigens (followed by the scratching) would be expected in atopic dermatitis patients. The high per-

centage（10－47%）of T cell clones specific for a dust mite antigen grown from lesional atopic dermatitis skin[9] could be explained by antigen-induced enrichment of the antigen specific T cells.

Conclusion

Atopic dermatitis was described in terms of stratum corneum barrier function. This is obviously an over-simplification. Many other factors should be considered, especially in terms of immunological dysfunction such as hyperstimulatory Langerhans cells and B-cell IgE overproduction[3]. A recent genetic analysis suggests that atopic dermatitis might be distinct from other atopic respiratory disorders（asthma and rhinitis）in that it is not associated with chromosome 11q[2]. Genetic linkage analyses have shown that atopic respiratory disorders are associated with chromosome 11q, where, interestingly, the β-subunit gene of high affinity IgE receptor is located[28].

Atopic dermatitis is a clinical entity and as such difficult to define. Further studies would be required to understand the nature and pathophysiology, of atopic dermatitis. The functional aspect of the stratum corneum, however, should not be ignored, because the stratum corneum is directly exposed to our environments and is the first defense against antigen penetration through the skin.

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