

Analysis of the Mechanism for the Development of Allergic Skin Inflammation and the Application for Its Treatment: Keratinocytes in Atopic Dermatitis — Their Pathogenic Involvement

Mayumi Komine^{1,*}¹Department of Dermatology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan

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Abstract. Atopic dermatitis frequently accompanies bronchial asthma, allergic rhinitis, and allergic conjunctivitis, the pathogenesis of which has frequently focused on the immunological aspects; however, skin eruption in atopic dermatitis occurs mainly in the epidermis, whose barrier function and cytokine expression have been revealed to be abnormal. In addition, the epidermis contains Langerhans cells, antigen-presenting cells, which could be considered the sentinel of the immune system. Some atopic dermatitis patients have been revealed to have mutations or SNPs (single-nucleotide polymorphisms) in the filaggrin gene, which affect the epidermal barrier function. Proteinases in the epidermis are of importance in maintaining the epidermal barrier, abnormalities of which have been reported in atopic dermatitis. Abnormalities of various cytokines and chemokines produced by keratinocytes have also been reported. Thymic stromal lymphopoietin (TSLP) produced by keratinocytes has recently been a focus in atopic dermatitis. Adrenergic/cholinergic responses in the epidermis could also influence the pathogenesis of atopic dermatitis. Considering epidermal keratinocytes as a trigger of immune abnormalities, not only as a peripheral effector, would be important to further disclose the pathogenesis of this enigmatic disorder.

Keywords: keratinocyte, atopic dermatitis, epidermal barrier, cytokine/chemokine, proteinase, allergic inflammation

Introduction

Atopic dermatitis is characterized by eczematous changes in the epidermis, and most patients have an atopic background with past and familial histories of bronchial asthma, allergic rhinitis, and/or allergic conjunctivitis. The serum IgE level is frequently elevated, but also many patients have normal levels of IgE, suggesting that atopic dermatitis is a heterogeneous disease entity. Table 1 shows the diagnostic criteria for atopic dermatitis established by the Japanese Society of Dermatology (2004) (1).

Histopathological changes of eczema occur mainly in the epidermis, that is, spongiosis of the epidermis (edema of the intercellular spaces of the epidermis),

infiltration of lymphoid cells into the epidermis and upper dermis. Thus, the epidermis is the main target of skin change in atopic dermatitis, suggesting that epidermal keratinocytes may be pathogenically involved in atopic dermatitis.

Table 1. Definition and criteria for atopic dermatitis by the Japanese Society of Dermatology (1)

Definition of atopic dermatitis:

Atopic dermatitis is an eczematous skin disease with a chronic clinical course with spontaneous waxing and waning, patients of which frequently have an atopic background.

Atopic background:

A familial history or past history of bronchial asthma, allergic rhinitis, allergic conjunctivitis, and/or atopic dermatitis, and/or a high serum IgE level.

*Corresponding author. mkomine12@jichi.ac.jp

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Filaggrin: the epidermal barrier function

The skin of atopic dermatitis patients is “dry” and “rough”, suggesting disruption of epidermal barrier function. Ichthyosis vulgaris is characterized by dry and rough skin with fish-like scales and has been known to be frequently accompanied by atopic dermatitis (2). Recently, the mutation in the filaggrin gene found in ichthyosis vulgaris has also been demonstrated in atopic dermatitis patients (3). Filaggrin is a protein in the granular layer of the epidermis that plays an important role in the aggregation of keratin filaments to form a rigid marginal band in horny layers (stratum corneum), the indispensable stage in keratinocyte terminal differentiation. Mutation in the filaggrin gene causes decreased barrier function of the horny layer of the epidermis (4). The expression level of filaggrin has been known to be decreased in atopic dermatitis patients at both the protein and mRNA levels (5, 6). A genome-wide study also demonstrated that locus 1q21, which contains the filaggrin gene, was strongly related to atopic dermatitis (7). Palmer et al. (3) revealed that the frequencies of two point mutations causing abnormal filaggrin gene function were significantly higher in atopic dermatitis patients than in the normal population. They speculated that low barrier function of the skin causes inflammation, and easier allergen intake through the epidermis, leading to easier establishment of sensitization (8). Consequently, a Th2-type reaction prevails, causing up-regulation of the serum IgE level. In mice, ova albumin sensitization through the skin caused a Th2-type reaction and upregulation of the serum IgE level, leading to higher sensitivity in the respiratory tract (9). The skin barrier function decreases with excessive washing and scratching, which also exacerbates atopic dermatitis. Filaggrin gene mutation may be part of the genetic background of atopic dermatitis patients, causing decreased skin barrier function, and these individuals become vulnerable to environmental stimuli (Fig. 1).

Proteinases

Netherton syndrome is characterized by atopic dermatitis-like skin eruption, bamboo hair, and an allergic history such as bronchial asthma and allergic rhinitis. The cause of this syndrome has been shown to be a mutation in the gene serine peptidase inhibitor Kazal-type 5 (*SPINK5*), which encodes one of the proteinase inhibitors (10). The product of *SPINK5* is lympho-epithelial Kazal-type inhibitor (LEKTI), which inhibits one of the serine proteinases, the kallikreins (KLK). The relationship between proteinases and atopic dermatitis has been intensely investigated. Mice

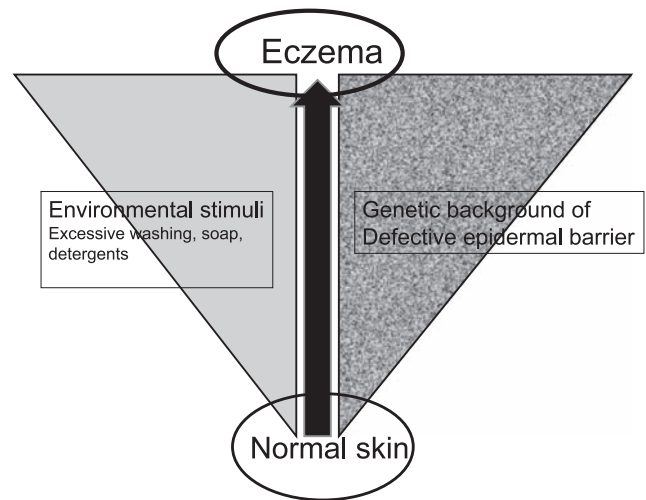


Fig. 1. Involvement of genetic predisposition and environmental factors in eczematous skin eruption. Genetic predisposition and environmental stimuli cooperate to develop eczematous changes in the skin.

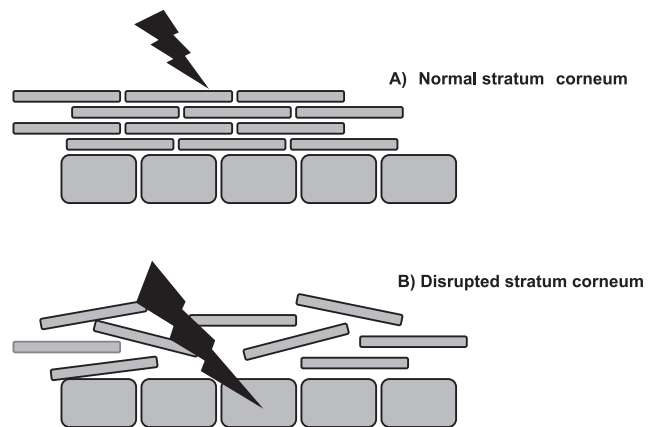


Fig. 2. Stratum corneum as a mechanical and immunological barrier of the epidermis. A: Normal stratum corneum protects living keratinocytes from environmental stimuli. B: Stratum corneum disrupted by excessive washing, detergents, or genetic background cannot protect living keratinocytes from environmental stimuli such as allergens.

expressing high levels of KLK5 or KLK7, both of which are targets of LEKTI, have been shown to spontaneously develop skin eruptions similar to atopic dermatitis (11). Most mite antigens, the exacerbating factors of atopic dermatitis, are also proteinases. In the epidermis, there are many kinds of proteinases and proteinase inhibitors, the balance of which is important to maintain proper skin barrier function (Fig. 2) (12).

Cytokines and chemokines

The epidermis is not only a physical barrier, but also

functions as a chemical and immunological barrier, producing various cytokines and antimicrobial peptides. In atopic dermatitis patients, cathelicidin (LL37) and β -defensin (HBD-2), antimicrobial peptides, are decreased, which is considered to be the cause of frequent cutaneous infections by staphylococcus and streptococcus (13).

Abnormalities of various cytokines have also been reported in atopic dermatitis patients. Serum levels of Th2 cytokines and chemokines, such as IL-4, IL-18 (14), and TARC/CCL17 (15), are elevated in atopic dermatitis patients; and mice expressing IL-4 or IL-18 in the epidermis spontaneously develop atopic dermatitis-like skin eruptions (16, 17). Mice expressing TARC/CCL17 in the epidermis did not spontaneously develop skin eruptions, but showed Th2-deviated skin inflammation in the contact hypersensitivity (CHS) reaction when stimulated repeatedly by FITC or oxazolone (18).

Recently, thymic stromal lymphopoietin (TSLP) has been reported to be highly expressed in the skin of atopic dermatitis patients. Mice expressing high levels of TSLP in the epidermis spontaneously developed atopic dermatitis-like skin eruptions (19). Dendritic cells (DC) stimulated by TSLP become inflammatory, expressing OX40 (CD134) ligand (OX40L), which triggers T cells to differentiate into inflammatory Th2-type cells, which express IL-4, IL-5, and TNF α (20). DCs stimulated by TSLP also produce IL-6, eotaxin-2/CCL24, MDC/CCL22, and TARC/CCL17. TSLP also directly stimulates mast cells to produce IL-5, IL-6, IL-13, and GM-CSF. These cytokines and chemokines induce allergic responses, IgE production, and eosinophilia (Fig. 3) (20). OX40 (CD134) is the receptor of

OX40L. Blocking the interaction of OX40 and OX40L has been shown to reduce symptoms in a mouse model of atopic dermatitis and bronchial asthma. Thus, anti-OX40L monoclonal antibody could be used in the treatment of atopic dermatitis and/or bronchial asthma (21).

We developed mice highly expressing TARC/CCL17 (18) or CTACK/CCL27 (22) in the epidermis utilizing keratin K14 promoter-driven TARC/CCL17 or CTACK/CCL27 constructs. These mice did not spontaneously develop skin eruptions; however, when we stimulated TARC/CCL17 transgenic (Tg) mice chronically with FITC or oxazolone, the contact hypersensitivity reaction was enhanced compared to that in control mice, and these mice showed an increased serum IgE level with intense mast cell infiltration into the skin. A similar phenomenon was observed in CTACK/CCL27 Tg mice with chronic stimulation with FITC. The epidermis of atopic dermatitis patients showed increased expressions of TARC/CCL17 (15) and CTACK/CCL27 (23). These results indicate that a higher expression of TARC/CCL17 and CTACK/CCL27 in epidermal keratinocytes in patients with atopic dermatitis would cause enhanced Th2-type immune responses with repeated antigen stimulation, working as a positive feedback loop in worsening the disease, which reflects the mechanism of induction of skin eruption by repeated antigen challenge in atopic dermatitis.

Adrenergic and cholinergic responses

Epinephrine has recently been revealed to be produced by epidermal keratinocytes, and its high affinity receptor, β_2 -adrenergic receptor, is also expressed in epidermal keratinocytes (24). β_2 -Adrenergic receptors are expressed in undifferentiated keratinocytes, but is suppressed in differentiated keratinocytes. A β_2 -adrenergic receptor antagonist has been reported to enhance epidermal barrier function. SNPs in the β_2 -adrenergic receptor are related to bronchial asthma and atopic dermatitis, and mutations in the β_2 -adrenergic receptor gene have been reported in atopic dermatitis or bronchial asthma patients (25, 26). These mutants inhibit the binding of the antagonist to the receptor (27). Maestroni et al. (28) revealed that β_2 -adrenergic receptor activation caused suppressed production of Th1 cytokines and their receptors, resulting in suppression of Th1 priming.

Acetylcholine is abundant in the epidermis, and its receptor is expressed in the nerve terminus of C fibers in the skin. Various cytokines, growth factors, and mechanical signals stimulate the production of acetylcholine in the epidermis, which suggests that acetylcholine signals inflammation to C fibers in the skin.

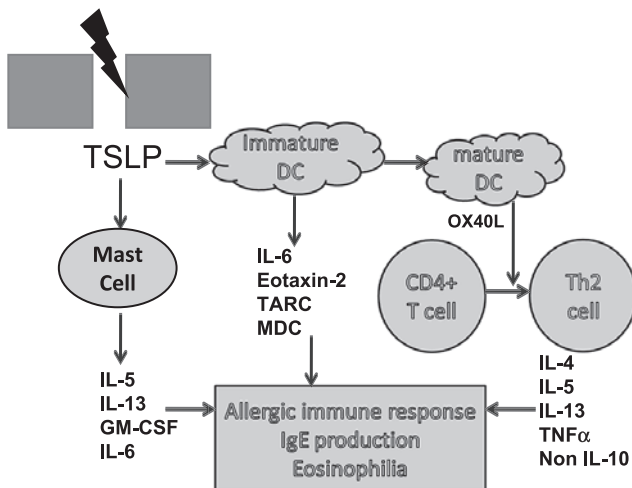


Fig. 3. Scheme implying that keratinocytes have a triggering role in atopic dermatitis. Environmental stimuli trigger keratinocytes to produce TSLP, which then activates downstream signals to enhance allergic immune responses.

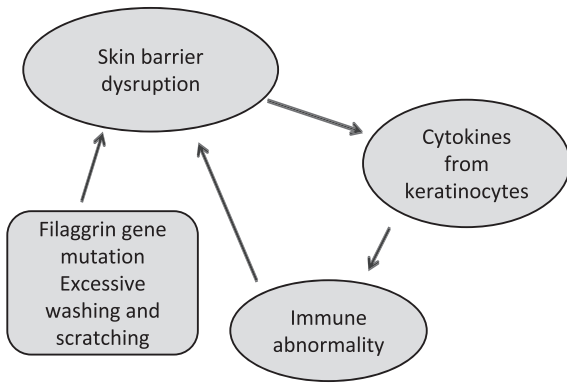


Fig. 4. Vicious cycle of allergic immune response. Disrupted skin barrier caused by excessive washing or genetic background induces production of inflammatory cytokines from keratinocytes, such as TSLP, which enhances the allergic immune response. Itch induced by the allergic immune response triggers scratching, which exacerbates skin barrier dysfunction.

The involved skin in atopic dermatitis patients has an elevated level of acetylcholine. Epidermis and upper dermis of atopic dermatitis patients contain 14 times more acetylcholine than normal subjects, and the deep dermis and subcutaneous fat of atopic dermatitis patients contain 3 times more acetylcholine than normal controls (29). Acetylcholine enhances sweating and itching and induces the dilation of blood vessels, wheals, and erythema, which could be deeply involved in the pathogenesis of atopic dermatitis.

Summary

These findings support the idea that keratinocytes are not the only peripheral effector reflecting pre-existing immune abnormalities, and enhancing skin inflammation, but also work as a trigger of an enhanced immune response or cause a Th1/Th2 imbalance to produce immunological abnormality. Atopic dermatitis patients have a genetic background that tends to produce a vulnerable skin barrier, leading to a Th2-polarized immune response and skin inflammation (Fig. 4). Considering atopic dermatitis not only as a disease of immunity and allergy, but also as a disease of a disrupted epidermal barrier, would help establish a novel therapeutic strategy for this enigmatic skin disorder.

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