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REVIEW ARTICLE

Pathomechanism of atopic dermatitis in the perspective of T cell subsets and skin barrier functions − "Which comes first, the chicken or the egg?"

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A B S T R A C T

Atopic dermatitis (AD) is a common skin condition that is characterized by a complex, heterogeneous pathogenesis. The possible candidates for its pathogenesis include skin barrier abnormality and allergy/immunology aspects. It has long been asked, "Which comes first, the barrier dysfunction or the allergy/immunology abnormality?" Recently, direct evidence of a link between the incidence of AD and loss-of-function mutations in the gene encoding *Filaggrin* has been discovered. This finding suggests that barrier dysfunction is a primary cause of AD. It has also been widely recognized that T cells play an important role in the development of AD in the perspective of the Th1/Th2 paradigm. Recently, however, new T cell subsets, Th17, T22, and regulatory T cells have been identified. In this review, we will update the roles of T cell subsets in AD and ascertain how skin barrier abnormality and allergy/immunology interact in a highly interdisciplinary manner.

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Introduction

Atopic dermatitis (AD) is a relapsing chronic inflammatory skin disease characterized by eczematous skin lesions and intense pruritus.[1] AD is one of the most frequent chronic inflammatory skin diseases and is increasingly prevalent, affecting at least 15% of children and 2−10% of adults in industrialized countries.[2] The skin is an active organ of the immune system and can, therefore, influence systemic immunity[3]; consequently, patients with AD often have other allergic disorders, such as food allergy, asthma, and allergic rhinitis,[2] which is called allergic (or atopic) march (Figure 1).[4] Therefore, it is important to evaluate the mechanism of AD. Thus far, the pathogenesis of AD has been attributed to a complex interaction among the environment and host susceptibility genes, altered skin barrier function and the immune system.[5] In this review, we will focus on the role of barrier functions and immune systems, especially in line with helper T (Th) cell subsets and regulatory T cells (Treg), in the development of AD.

Barrier

Outermost barriers are critical to avoid desiccation and to protect us from insult from foreign bodies. Mammalian skin consists of two sets of barriers: the stratum corneum and tight junctions (TJs). Thus far, several causes of xerosis have been considered: (i) a decrease in skin ceramides[6]; (ii) alterations of the stratum corneum pH[7]; (iii) overexpression of the proteases, including kallikreins (KLKs) and chymases[8]; and (iv) defects in *Filaggrin* (*FLG*).[9]

In Netherton syndrome, unregulated pH-sensitive KLK5 directly activates proteinase-activated receptor 2 and induces nuclear factor kappaB-mediated overexpression of thymic stromal lymphopoietin (TSLP), which aggravates AD-like skin lesions.[10]

The role of TJ in AD remains unknown. A knockout study of claudin-1, a TJ-specific integral membrane protein, demonstrated that TJs function as paracellular diffusion barriers in mammalian epidermis.[11] In humans, lack of claudin-1 lead to ichthyosis with scalp hypotrichosis, scarring alopecia, neonatal sclerosing cholangitis, and leukocyte vacuolization (NISCH syndrome),[12] but a precise description in terms of skin manifestation has not been provided, especially in relation to AD.

One of the characteristics of AD is dry skin that affects both lesional and non-lesional skin areas.[1] Dry skin in AD is in accord with increased transepidermal water loss, which suggests that the skin barrier is disrupted in AD. It has long been thought that the

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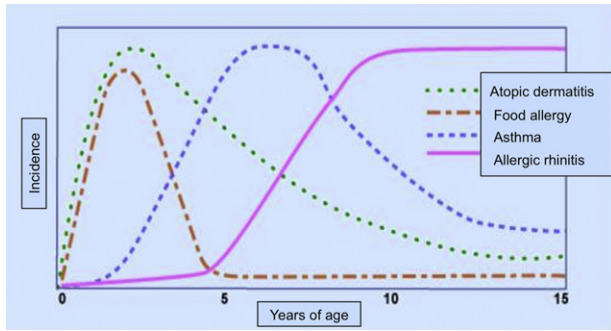
Figure 1 Allergic march. The allergic march is characterized by a typical sequence of sensitization and manifestation of symptoms that appear during a certain age period, persist over years or decades, and often show a tendency for spontaneous remission with age.

barrier abnormality in AD is not merely an epiphenomenon but rather the initiator of its pathogenesis.[13] As a result of the barrier disruption, the skin may permit the penetration of external stimuli, such as allergens, bacteria, and viruses.[14]

Role of Filaggrin in skin barrier

The direct evidence for a primary structural abnormality of the stratum corneum in AD is a recently discovered link between the incidence of AD and loss-of-function mutations in the gene encoding *FLG*. Individuals carrying the *FLG* null allele variants tend to develop AD.[9] FLG protein is localized in the granular layers of the epidermis (Figure 2). Profilaggrin, a 400-kDa polyprotein, is the main component of keratohyalin granules.[15,16] In the differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into 10–12 FLG molecules (a molecular mass of 37 kDa in human and 27 kDa in mice), which aggregates in the keratin cytoskeleton system to form a dense protein-lipid matrix in humans.[15] These FLG monomers are further degraded into natural moisturizing factors by either caspase 14 or bleomycin hydrolase, which are important for maintaining hydration and keeping the skin pH low (Figure 2). Intriguingly, it has recently been reported that intragenic copy

number variation (20–24 copies in one person) within a *FLG* gene contributes to the risk of AD with a dose-dependent effect.[17]

Flaky tail (*Flg^ft*) mice, essentially deficient in Filaggrin, have been used to investigate the role of Filaggrin in AD.[18] There have been four recent studies using *Flg^ft* mice as a model of Filaggrin deficiency: Fallon et al[19] used *Flg^ft* mice from which the *ma* mutation had been eliminated with four additional backcrosses to B6 mice, and others used commercially available *Flg^ft* mice.[20–22]

The first report showed only a histological abnormality without clinical manifestations,[19] the second report demonstrated spontaneous eczematous skin lesions after 28 weeks of age,[20] the third report did not indicate any spontaneous dermatitis in *Flg^ft* mice,[21] and the fourth report observed spontaneous dermatitis as early as 5 weeks of age, with gradual exacerbation with age.[22] Humans and mice differ in this respect, since most AD resolves with age in humans and the cutaneous manifestations occurred only in homozygous *Flg^ft* mice. The discrepancies between these results seem to be related to the presence or absence of the *ma* mutation, variation in the genetic backgrounds of each mouse strain, and environmental factors.

Human AD can be categorized into the extrinsic and intrinsic types. Extrinsic or allergic AD shows high total serum IgE levels and the presence of specific IgE for environmental and food allergens, whereas intrinsic or non-allergic AD exhibits normal total IgE values and the absence of specific IgE.[23] The skin barrier is perturbed in the extrinsic, but not the intrinsic, type. *FLG* gene mutations are not a feature of extrinsic, but not intrinsic, AD.[24]

Dendritic cells as an initiator for skin immune responses

Dendritic cells (DCs) play an important role in the initiation of acquired immune responses. In the skin, there exist two populations of DCs; epidermal Langerhans cells (LCs), and dermal DCs. According to their expression of Langerin, dermal DCs are divided into at least two populations: Langerin-positive dermal DCs and Langerin-negative dermal DCs.[25–27] LCs have long been regarded as essential antigen presenting cells for the establishment of sensitization in hapten induced-contact hypersensitivity, but this concept is now being challenged by recent analyses using LC ablation murine models.[28] Langerin-negative DCs play a major role in the
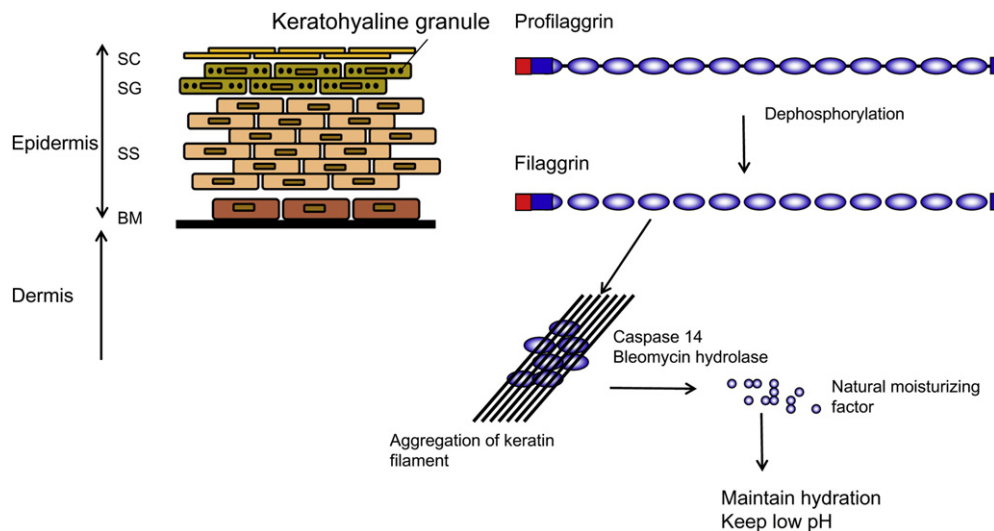
Figure 2 Role of Filaggrin. FLG protein is localized in the granular layers of the epidermis. Profilaggrin is the main component of keratohyaline granules. In the differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into 10-12 FLG molecules, which aggregates in the keratin cytoskeleton system to form a dense protein-lipid matrix. These FLG monomers are further degraded into natural moisturizing factors, which are important to maintain hydration and keep the skin pH low.

development of contact hypersensitivity, and LCs and Langerin-positive dermal DCs play a compensatory role.[29]

Conversely to hapten, which is a contact sensitizer that is small enough to penetrate into the dermis of the skin, allergens for AD are rather large. Therefore, LCs are thought to be the DCs responsible for the acquisition of cutaneous allergens, such as house dust and mites, in the development of AD. It has been demonstrated that activation-induced LCs elongate their dendrites to penetrate tight junctions and monitor the extra-tight junction environment located outside of the tight junction barrier.[30]

Consistent with the above finding, in a mouse model of AD induced by a vitamin D3 analogue, epidermal LC-depleted mice treated with vitamin D3 did not exhibit either AD-like inflammation or increased serum IgE, as compared to control mice treated with the vitamin D3 analogue.[31] Overexpression of TSLP by keratinocytes results in an AD-like inflammatory phenotype in mice,[32] and TSLP is highly expressed in the epidermis of AD patients.[33] In addition, by applying an LC ablation system, it has recently been reported that LCs are crucial for Th2 induction and IgE production through TSLP signaling upon epicutaneous protein exposure.[34] These results demonstrate that LCs are required for the development of AD in mouse models of AD through epidermal TSLP overexpression.[31]

Th1−Th2 paradigm in AD

AD has been considered the paradigm of an allergic Th2-mediated disease, which is characterized by abnormal IgE production, peripheral eosinophilia, mast cell activation, and induction of Th2 lymphocytes expressing interleukin (IL)-4, IL-10, and IL-13.[1]

Consistently, in mice, it has been established that repeated application of hapten (2,4,6-trinitrochlorobenzene; TNCB) induces AD-like skin lesions.[35] In this model, whilst a single challenge induces contact hypersensitivity, a prototype of delayed-type hypersensitivity, repeated hapten application results in a shift from Th1- to Th2-mediated cutaneous inflammation, with elevated IL-4 expression, eosinophil infiltration in the skin, and elevated hapten-specific serum IgE levels.[35] In addition, extraordinary sustained interferon (IFN)-γ expression leads to a Th1-dominant state in the mouse AD model, with clinical amelioration of AD symptoms.[36]

It is known that epicutaneous application of protein antigen, ovalbumin (OVA), induces a rise in OVA-specific serum IgE and IgG1, both of which are induced in a Th2-dependent manner, as well as the development of dermatitis, characterized by the infiltration of CD3+ T cells, eosinophils, and neutrophils and local expression of mRNA for the cytokines IL-4, IL-5, and, intriguingly, IFN-γ.[37] Consistently, chronic exposure to protein antigens, especially those with protease activities, such as house dust mite allergens, induces TSLP expression in the epidermis. The above findings suggest that chronic exposures to either hapten or protein antigen induce AD-like skin lesions in mice, which fits well with the pathogenesis of human AD (Figure 3).

By contrast, in a patch test, sequential biopsies in AD patients exposed to aeroallergens, such as house dust mite, demonstrated a biphasic immunologic response characterized by a switch from a Th2 toward a Th1 phenotype in later phases of the disease.[38] It remains unknown how a Th1 shift occurs in the clinical setting. The Th2 environment is known to inhibit the production of antimicrobial peptides in the skin, which predispose the skin to superimposed infection.[39] Therefore, one possible explanation for Th2 to Th1 shifting in the chronic phase of AD is that superimposed infection of *Staphylococcus aureus* shifts the skin condition towards Th1. Intriguingly, microbial colonization by *S aureus* amplifies inflammation, and further stresses the barrier in AD. It is important to address the role of Th1 shifting in the pathogenesis of AD in the future.
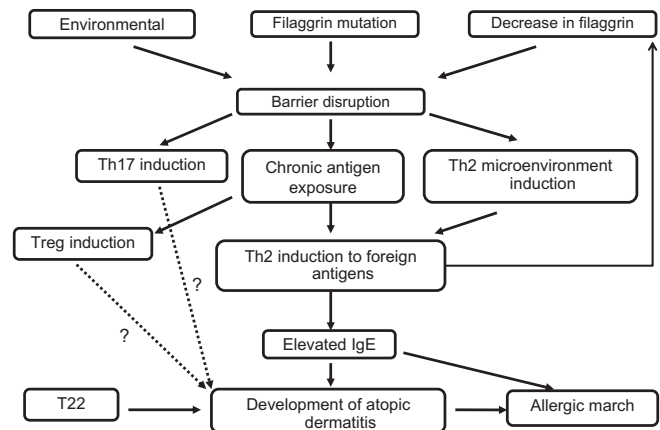
Figure 3 Scheme on the development of AD. As shown in the figure, both barrier and allergy/immunology play an important role in the development of AD in a highly interdisciplinary manner.

Th17 in the development of AD

In addition to the Th1/Th2 paradigm, the roles of Th17 and regulatory T cells (Treg) have been highlighted.[40−43] Transforming growth factor (TGF)-β, IL-1β, IL-6, and IL-23 are key factors involved in naive T cell commitment to a Th17 phenotype. Th17 cells are characterized by the production of inflammatory cytokines such as IL-17A, IL-17F, IL-22, and IL-26. Th17 cells have been implicated in several human autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and psoriasis.[44]

Epicutaneous immunization in mice with ovalbumin can cause allergic skin inflammation resembling AD, cutaneous expression of IL-17A and IL-17F mRNA and, subsequently, airway inflammation[45] (Figure 3). In addition, the presence of Th17 cells and IL-17 in AD patients has been demonstrated. Phenotypic analysis of peripheral blood mononuclear cells derived from AD patients exhibited a marked increase in the IL-17+CD4+ T-cell population. The high percentage of IL-17-producing cells was found in severe AD,[46,47] which suggests a direct correlation between the presence of Th17 cells and the severity of the disease. Increased levels of IL-17 were also reported in cutaneous lesions of AD patients. Consistently, *Flg*[ft] mice were shown to possess increased IL-17 mRNA levels in skin lesions shortly after epicutaneous application of OVA.[20] It has been demonstrated that IL-17A and IL-17F promote eosinophil production of CXCL1, IL-8, and CCL4, as well as IL-1β and IL-6, indicating that there is possible crosstalk between Th17 cells and eosinophils in AD.[48]

By contrast, distinct IL-22-producing CD4+ and CD8+ T-cell populations (called T22) were significantly increased in AD skin. IL-22+CD8+ T-cell frequency correlated with AD disease severity.[49] Therefore, the above findings suggest that a Th2/T22 polarization dominates chronic AD. CCL27, the ligand for CCR10, is highly expressed in skin lesions from AD patients,[50] and, combined with the recent finding that T22 cells particularly express CCR10, this might be an explanation for the increased presence of T22 in chronic AD skin. IL-22 has been shown to induce acanthosis and hypogranulosis in skin by downregulation of genes involved in terminal differentiation of the skin,[51] which may be involved in the development of AD (Figure 3).

Treg in the suppressor for AD

Treg exist in all non-lymphoid tissues, and the skin is one of the tissues that has a high proportion of Treg in the steady state in both mice and humans.[43,52,53] Treg in the skin are CD44+ and CD103^{high} [43,52,53], and they express chemokine receptors, CCR4,

CCR5, CCR6 and CCR7. CCR5⁺ Treg preferentially migrate to cutaneous lesions of *Leishmania major* infection.[54] Mice with a complete loss of CCR4 on Treg develop spontaneous lymphocytic infiltration and severe inflammation in the skin and lungs, accompanied by peripheral lymphadenopathy and increased differentiation of skin tropic CD4⁺Foxp3⁻ memory T cells. Using α-1, 3-fucosyltransferase VII (Fut7) deficient mice, Dudda et al have reported the importance of E- and P-selectin ligands for Treg migration to the skin.[53] Loss of the bindings of selectins caused skin specific inflammation, indicating the essential role of skin-resident Treg for maintaining immune homeostasis locally.

In addition, it has also been reported that Treg is accumulated in the skin during contact hypersensitivity[43] and that IL-10 is induced in the repeat hapten application-induced chronic contact hyper-sensitivity model,[55] indicating that chronic antigen exposure induces Treg accumulation into the skin (Figure 3).

From observation of patients with IPEX syndrome (immuno-dysregulation polyendocrinopathy enteropathy X-linked syndrome) who show atopic-like dermatitis and high IgE levels as well as loss of Treg functions, it appears likely that Treg is related to the development of AD lesion.[56] AD patients have a higher number of Treg in peripheral blood with comparable suppressive activity[57,58] and expansion of Treg has been positively associated with disease activity in AD.[59] On the contrary, it has also been reported that the numbers of Treg in peripheral blood are similar in both AD patients and healthy controls.[60] Although it was initially reported that Treg were absent in the AD skin lesion, whereas Tr1 were detected,[61] several groups later reported the existence of Treg in the skin lesion of AD.[62,63] Reefer et al, however, reported that a new subtype of Treg with Th2 promoting ability was observed in AD and that its functions depended on the expression of CCR6.[64] AD is a chronic inflammatory disease that has multiple disease stages, and treatments for AD, such as cyclosporine,[58,60,65] glucocoritoid,[66] and UV[67] can alter the number of Treg in the blood. Therefore, the interpretation and comparison of each study need careful attention.

Barrier and allergy/immunology interplay in the development of AD

It has been investigated whether acute removal of stratum corneum modulates the production of cytokines and chemokines by epidermal cells. Tape stripping upregulates TSLP levels in the skin, which polarizes skin DCs to elicit a Th2 response via the induction of IL-10.[68] Therefore, barrier disruption itself seems to shift the skin environment towards Th2. In addition, Th2 chemokines (CCL17 and CCL22) and eosinophil chemoattractant (CCL5) mRNA levels were remarkably elevated in mice by barrier disruption, which was augmented by tape-stripping more markedly than by acetone-rubbing.[69] Furthermore, tape stripping induced dermal infiltration of eosinophils in mice.[69] These findings suggest that acute barrier removal induces the expression of Th2 and eosinophil chemokines by epidermal cells and easily evokes the late phase reaction upon challenge with antigen. This indicates that the barrier dysfunction predisposes the skin environment to Th2 skewing conditions as well as the feasibility for antigen exposure to the internal skin (Figure 3). Therefore, this conclusion answers the initial question, "Which comes first, the chicken or the egg?" Under certain conditions, especially when the Filaggrin is mutated or scratching disrupts the skin barrier, the barrier dysfunction is the primary cause of the development of AD.

Intriguingly, by contrast, human keratinocytes differentiated in the presence of IL-4 and IL-13 exhibited significantly reduced *FLG* gene expression.[70] In addition, IL-17A downregulates the expression of Filaggrin and genes important for cellular adhesion, which leads to the impairment of epidermal barrier formation.[71] Consistently,

the level of FLG expression in AD patients, even those without *FLG* mutations, was decreased. These findings indicate that the atopic inflammatory response can induce an acquired barrier defect and that these create a positive feedback loop by interplaying in a highly interdisciplinary manner in the development of AD.

Declaration of all sources of funding

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