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Significance of Skin Barrier Dysfunction in Atopic Dermatitis

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The epidermis contains epithelial cells, immune cells, and microbes which provides a physical and functional barrier to the protection of human skin. It plays critical roles in preventing environmental allergen penetration into the human body and responsing to microbial pathogens. Atopic dermatitis (AD) is the most common, complex chronic inflammatory skin disease. Skin barrier dysfunction is the initial step in the development of AD. Multiple factors, including immune dysregulation, filaggrin mutations, deficiency of antimicrobial peptides, and skin dysbiosis contribute to skin barrier defects. In the initial phase of AD, treatment with moisturizers improves skin barrier function and prevents the development of AD. With the progression of AD, effective topical and systemic therapies are needed to reduce immune pathway activation and general inflammation. Targeted microbiome therapy is also being developed to correct skin dysbiosis associated with AD. Improved identification and characterization of AD phenotypes and endotypes are required to optimize the precision medicine approach to AD.

Key Words: Atopic dermatitis; epidermal barrier; antimicrobial peptide; microbiome; moisturizer

INTRODUCTION

Atopic dermatitis (AD) is the most common chronic skin disease worldwide.[1,2] It affects about 20% of children and 5% of adults.[1,3-5] Patients with persistent or severe AD suffer from profound impairment of their quality of life.[2,6,7] Additionally, AD places a heavy economic burden on patients and their family.[8,9] AD is strongly associated with the development of food allergy, bronchial asthma, and allergic rhinitis, commonly referred to as the Atopic March.[10-15] The epidermis provides a physical and functional barrier to the human body, and skin barrier defects are the most important pathologic findings in AD skin.[16-18] Skin barrier defects have been considered an initial step in developing AD.[4,17] Recently, investigators have demonstrated that multiple factors, including immune dysregulation, defects in terminal epithelial differentiation such as lack of filaggrin (FLG), deficiency of antimicrobial peptides (AMPs), altered composition of stratum corneum intercellular lipids, and altered skin microbiome may affect skin barrier function (Fig. 1).[2,4,16,19,20] These factors interact with each other and may modify skin barrier function. In this review, we discuss normal skin barrier and pathogenesis of skin barrier defects associated with the development of AD skin disease. Additionally, we review the role of emollients, anti-inflammatory agents, sodium hypochlorite, probiotics, and microbiome in the treatment and prevention of AD development. Moreover, various types of immune-directed targets for biologic therapy are reviewed.

Normal skin barrier

The skin barrier plays a critical role in preventing allergen and microbial penetration into the human body.[4,10,21] The epidermis consists of a 15- to 30-nm-thick layer of proteins and lipids, and provides a physical and functional barrier to the human body.[22,23] The physical skin barrier is mainly localized to the uppermost area of the epidermis which is the cornified layer (stratum corneum).[22,24] The epidermis is continuously regenerated by terminally differentiating keratinocytes, which is known as cornification or keratinization.[22,23] Cornification begins with the migration of keratinocytes from the basal to upper layers, and ends with the formation of the cornified layer.[22,23] During epidermal differentiation, lipids are produced by keratinocytes and extruded into the extracellular space to form extracellular lipid-enriched layers.[22-24] Omega-hydroxy-ceramides are covalently bound to cornified envelope proteins and form the backbone for the subsequent addition of free ceramides, free fatty acids, and cholesterol in the cornified layer.[22-24] The epidermis undergoes complete turnover every 28 days.[25]

Cell proliferation, differentiation, and death occur sequential-

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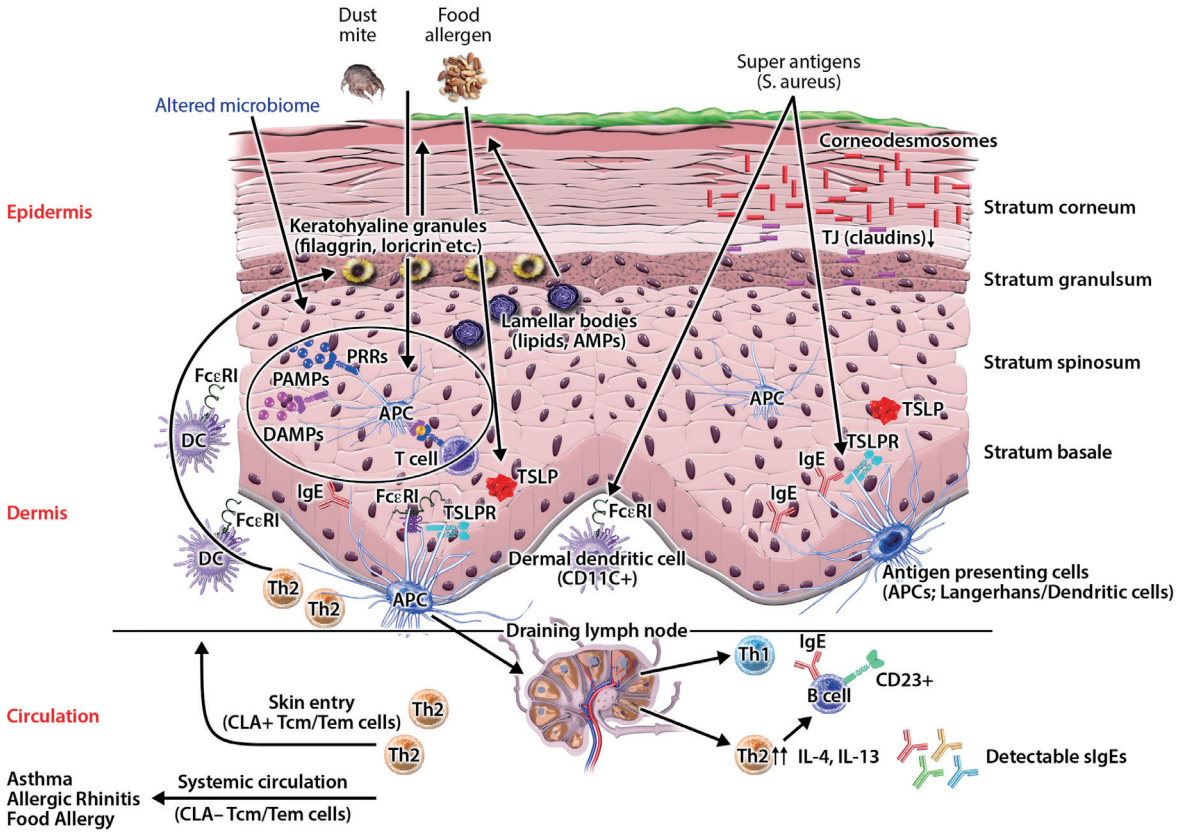

Fig. 1. Impaired skin barrier enhances allergen penetration and activates the innate immune system. Multiple factors, including immune dysregulation, defects in terminal epithelial differentiation such as lack of filaggrin (FLG), deficiency of antimicrobial peptides (AMPs), altered composition of stratum corneum intercellular lipids, and altered skin microbiome cause skin barrier defects. Source: Czarnowicki *et al.* J Allergy Clin Immunol 2017;139:1723-34.

ly, and each process is characterized by the expression specific proteins, including occludin, claudins, keratins, transglutaminases (TGs), loricrin, and FLG.[22,23,26,27] Keratinocytes express specific differentiation markers according to their stage of epidermal differentiation.[22] For instance, keratin 5 and TG2, which are expressed in the basal layer, represent early differentiation markers. In contrast, FLG, which is expressed in the upper granular and cornified layers, is a late differentiation marker. Tight junctions (TJs), desmosomes, and adherens junctions are paracellular proteins that form a permeability barrier between adjacent cells and involve cell adhesion.[26-29]

Keratinocytes also produce AMPs including cathelicidin (LL-37) and beta-defensins (HBDs), which kill microbes and play important roles in maintaining skin homeostasis.[30,31] In addition to their antibacterial activity, AMPs kill viruses and fungi through multiple modes of action.[31] The levels of AMPs, such as HBDs and LL-37 in epidermis, are low in normal health conditions, but are highly expressed upon infection and inflammation.[31,32] AMPs form an innate epithelial chemical barrier and have pleiotropic functions.[31,33] They not only kill microbes, but also control inflammation and regulate the skin barrier.[31,34,35] Impaired TJ protein expression contributes to skin barrier dysfunction in AD.[36] HBD-3 improves the function of the epithelial TJ barrier by inducing expression of several claudins.[34] HBDs and LL-37 also induce production of IL-18 through p38 and Erk mitogen-activated protein kinase activation in human keratinocytes.[37] Additionally, they induce expression of IL-6, IL-10, macrophage inflammatory protein-3 alpha, and RANTES.[38] Furthermore, it has been reported that HBDs and LL-37 induce keratinocyte migration, proliferation, re-epithelialization, neovascularization, and wound healing.[31,35,38,39]

The cornified layer is surrounded by a continuous lipid matrix which provides a barrier against water and prevent water loss.[24,40,41] The lipid matrix mainly consists of 3 lipid classes: cholesterol, free fatty acids, and ceramides.[23,42] Therefore, the lipid matrix in the cornified layer may play crucial roles as a part of skin barrier and shows altered composition in AD skin.

It has also recently been reported that the epidermal microbiome may also play crucial roles in maintaining skin barrier function.[4,16,43] Previously, the biogeography of the skin microbiome has been reported in children and adults.[44,45] Several studies have shown that human skin microbiome is site-specific.[44-46] Recently, it has been reported that the gut and cutaneous commensal bacteria, including *Staphylococcus* (*S.*) *epidermidis*, and

S. hominis, play important roles in skin homeostasis and host defense against microbial penetration.[47-50]

Dysregulation of the skin barrier in AD

Epidermal barrier proteins, including FLG, TGs, keratins, loricrin and intercellular proteins, are cross-linked to form an impermeable skin barrier.[22,23] Skin barrier defects facilitate allergen sensitization and lead to systemic allergic responses, such as increased IgE levels and airway hyperreactivity.[36,51-53] Transepidermal water loss (TEWL) is a noninvasive measurement used to evaluate skin barrier function.[54] Patients with AD have increased TEWL, which reflects skin barrier dysfunction in AD, and can precede clinical AD.[55,56]

AD skin is characterized by overexpression of Th2 and Th22 cytokines that contribute to skin barrier dysfunction by altering protein and lipid content in the skin (Table 1).[2,4,57,58] FLG is a key epidermal barrier protein.[22,59] It is degraded into free amino acids and these amino acids are essential for maintaining skin pH and the retention of water contributing to osmolarity in the cornified layer.[60-62] FLG deficiency alters the shape of corneocytes in the skin and enhances skin inflammation by facilitating epicutaneous sensitization in murine models of eczema.[41,63] FLG deficiency also causes paracellular skin barrier abnormality that reduces inflammatory thresholds to irritants and haptens.[41,64] FLG proteolysis occurs upon exposure to a low humidity environment and can be prevented by high humidity.[65] FLG is decreased in AD skin by overexpression of IL-4, IL-13, IL-25, IL-17A, and IL-22 (Fig. 2).[57,59,66,67] Additionally, loricrin, and involucrin, which are major epidermal barrier proteins, are also down-regulated by Th2 cytokines through STAT6 signaling in AD skin.[68] It is well known that FLG mutation is a major predisposing factor for AD development, particularly in patients who have early-onset AD and those with persistent AD.[21,55,69-72] However, a significant number of AD patients do not have any type of FLG gene mutation, and about 40% of individuals with FLG-null alleles do not have AD.[21,73] Moreover, most of the patients with AD and FLG mutations eventually recover from AD.[21,73,74] Therefore, FLG mutations contribute to AD, but in isolation it is not sufficient to generate AD. There are other factors that result in AD development. Intercellular proteins, including TJs, desmosomes, and adherens junctions, form a permeability barrier between adjacent cells and aids with cell adhesion.[26-29] Th2 cytokines down-regulate TJs, and impaired TJs contribute to abnormal skin barrier function in AD.[36,75] Corneodesmosin (CDSN) is an intercellular protein that plays a critical role in maintaining skin barrier function.[29,76] Recently, Lee *et al.*[77] have reported that CDSN expression is down-regulated by cytokines, including IL-4, IL-13, IL-22, IL-25, and IL-31. Additionally, CDSN deficiency resulted in lethal-skin barrier disruption in a mouse model,[76] and enhanced viral penetration in an organotypic skin model.[77] Therefore, a variety of cytokines modulate epidermal barrier proteins, and cause skin barrier defects.

AMPs, such as LL-37 and HBD-3, are highly expressed by keratinocytes during infection, inflammation, and wounding.[30,31] AMPs form an innate multidimensional epithelial chemical barrier.[31,33] They not only have antimicrobial activities, but also

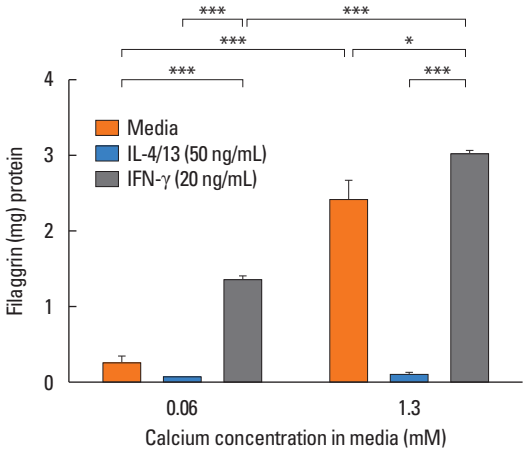

Fig. 2. Keratinocytes differentiated in the presence of IL-4 and IL-13 exhibit significantly reduced filaggrin. Primary human keratinocytes were cultured for 5 days in 0.06 or 1.3 mmol/L $CaCl_2$ in the presence of IL-4 plus IL-13 or interferon (IFN)-gamma. $*P<0.05$; $***P<0.001$ between the exposure groups. Source: Howell *et al.* J Allergy Clin Immunol 2007;120:150-5.

Table 1. Epidermal Barrier Dysfunction in atopic dermaitis

Epidermal Barrier	Abnormalities	Functional effects	References
Terminal epithelial differentiation products	Reduced filaggrin, loricrin, involucrin, corneodesmosin, keratin 1 and 10.	Decreased skin water content, enhanced allergen, microbial penetration, and increase skin pH.	32, 60, 68, 77, 82
Tight junctions	Decreased claudin-1, 8, and 23.	Increased transepidermal water loss (TEWL), enhanced allergen and microbial penetration, and decreased cohesion.	36, 75
Microbial barrier	Cutaneous dysbiosis	Skin inflammation, microbial skin infections, keratinocytes death, and exacerbation of AD.	3, 49, 81, 94
Lipids	Altered compostion of epidermal lipids and decreased ceramide.	Staphylococcal infection, dry skin, and increased TEWL.	4, 86, 88
Immune barrier	Decreased cathelicidin, HBD-2, and HBD-3.	Recurrent microbial infections, skin dysbiosis, and exacerbation of AD.	4, 30, 31, 33

regulate the skin barrier.[31,34,35] AMP expressions are inhibited in AD skin by Th2 cytokines, which are overexpressed in AD skin.[32,78-80] The deficiency of AMPs and over-expressed Th2 cytokines in AD skin is associated with a higher propensity to *S. aureus* infection, which is known to play critical roles in the exacerbation of AD.[30,31,81] Son *et al.*[82] have reported that *S. aureus* inhibited expression of terminal differentiation markers, including FLG, loricrin, and keratina 1 and 10. Recently, Brauweiler *et al.*[83] have also demonstrated that *S. aureus* lipoteichoic acid inhibits keratinocyte differentiation markers, including keratins 1 and 10, and desmocollin1, through a p63-mediated pathway. Therefore, deficiency of AMPs and overexpressed Th2 cytokines in AD skin may lead to frequent microbial skin infections and skin barrier defects.[32,84,85]

AD skin also has a defective lipid matrix. This causes impaired skin barrier function.[18,86,87] Stratum corneum intercellular lipid composition in AD skin is characterized by altered expression of enzymes involved in the biosynthesis of free fatty acids and ceramides.[86,88] Researchers have demonstrated that altered composition of stratum corneum intercellular lipids correlates with *S. aureus* colonization status in AD.[89] Additionally, it has been reported that a synthetic omega-hydroxyceramides enhanced the integrity of the stratum corneum, and accelerated the recovery of damaged skin barrier function by stimulating differentiation processes.[90] Lowe *et al.*[91] also reported that routine lipid replacement reduced the incidence of AD during the active treatment period by approximately fifty percent. Therefore, the lipid matrix in the cornified layer may play a crucial role as part of the skin barrier.

Microbiome

AD is associated with abnormal skin colonization of pathogens, such as *S. aureus*.[4,92] Commensal bacteria induce AMPs and inhibit *S. aureus* on the human skin.[16] In contrast, cutaneous dysbiosis affects skin immune responses and causes skin inflammation.[49,93,94] Moreover, skin dysbiosis may cause skin barrier defects.[95,96] Species-level investigation of AD flares demonstrated greater *S. aureus* predominance in patients with more severe disease, and *S. epidermidis* predominates in patients with less severe disease.[49] Additionally, *S. aureus* isolates from AD patients with more severe flares induced epidermal thickening and expansion of cutaneous Th2 and Th17 cells.[49] However, Kennedy *et al.*[97] reported that commensal *staphylococci* were significantly less abundant in infants with AD. This finding suggests that commensal bacteria might protect against the development of AD.

AMPs, such as HBD-3 and LL-37, are highly expressed after various exposures in the normal healthy skin.[31] Down-regulated AMPs by Th2 cytokines in AD skin causes recurrent microbial infections and may affect skin pH.[41,85,98] Several factors, including FLG, cytokines, proteases, enzymes, and microbes, alter skin pH.[42,98,99] Skin pH is an important factor controlling skin homeostasis. Increased skin pH also facilitates microbial skin infections and skin barrier defects.[4,98,99] Additionally, Brauweiler *et al.*[100] have demonstrated that staphylococcal alpha toxin, a primary toxin of *S. aureus*, causes cell death and consequently skin barrier defects. Thus, decreased levels of AMPs may cause skin dysbiosis and skin barrier defects. In summary, the skin dysbiosis and deficiency of AMPs may affect skin homeostasis and cause skin barrier defects in AD skin.[4,16,47,49] However, additional studies are needed to elucidate how dysbiosis affects epidermal barrier function.

Clinical implications in the treatment of AD

Moisturizers, including petrolatum, physiological lipid mixtures, and ceramide-dominant triple-physiolosic lipid (ceramide: cholesterol:free fatty acids at a 3:1:1 molar ratio), play critical roles in AD management.[1,101,102] They improve clinical symptoms and skin barrier function, and reduces bacterial colonization.[4,102-107] Petrolatum improves skin barrier functions by up-regulation of AMPs, including LL-37, HBD-2, elafin, and S100 proteins.[101] Additionally, epidermal differentiation markers, such as FLG and loricrin, are induced by moisturizers.[4,101] Moreover, petrolatum significantly reduces T-cell and dendritic cell infiltration in AD skin.[101] Glatz *et al.*[108] have reported that early emollient therapy alters the skin barrier and microbes in high-risk newborns. Of note, Nakatsuji *et al.*[16] have demonstrated that application of coagulase-negative *Staphylococcus* strains to the skin of patients with AD decreases colonization by *S. aureus*.

It has been reported that use of dilute bleach (sodium hypochlorite) baths and intranasal mupirocin treatment improves AD symptoms.[109] Other investigators have reported that topical use of bleach inhibites *S. aureus* and show beneficial effects on AD skin possibly through intrinsic anti-inflammatory effects.[110,111]

Hyung *et al.*[112] reported *Lactobacillus* strain, CJLP55, isolated from kimchi, reduced infiltration of mast cells, eosinophils, and production of Th2 cytokines in AD-induced mouse skin. Additionally, Notay *et al.*[113] analyzed 315 articles and reported that probiotics and prebiotics improved AD symptoms including quality of life and clinical severity.

Recently, various types of immune therapy have been developed (Table 2). Clinical studies with broad and targeted therapies have been applied for patients with moderate-to-severe AD.[1] Cyclosporine and oral glucocorticoids have been used, but there are limitations due to multiple adverse reactions. Dupilumab, anti-IL-4 Rα monoclonal antibody, improved clinical findings in adults with moderate-to-severe atopic dermatitis, without significant safety concerns.[114-117] Additionally, dupilumab up-regulated genes involved in skin barrier function.[117] It will be interesting to learn if early treatment of AD with dupilumab could prevent progression of the atopic march.

Table 2. Recent controlled trails in patients with atopic dermatitis

Agent	Trade name	Target	Drug	Phase	Manufacturer	ClinicalTrials.gov
Dupilumab		IL-4Rα	Anti-IL-4Rα mAb	Phase III published	Regeneron	NCT01949311
Crisaborole		PDE4	Topical PDE4 Inhibitor	Phase III published	Pfizer	NCT02118766 NCT02118792
Ustekinumab	Stelara	IL-12/23p40	Anti-p40 mAb	Phase II published	Janssen	NCT01806662
Tralokinumab		IL-13	Anti-IL-13 mAb	Phase II completed	MedImmune	NCT02347176
Tofacitinib		JAK1/3	Topical JAK1/3 Inhibitor	Phase II published	Innovaderm	NCT02001181
Lebrikizumab		IL-13	Anti-IL-13 mAb	Phase II completed	Hoffmann-La Roche	NCT02340234
CIM331/Nemolizumab		IL-31R	Anti-IL-31R mAb	Phase II completed	Chugai	NCT01986933
QGE031		IgE	Anti-IgE mAb	Phase II completed	Novartis	NCT01552629
Apremilast	Otezla	PDE4	PDE4 Inhibitor - Oral small molecule	Phase II completed	Celgene	NCT02087943
QAW039/Fevipiprant		CRTH2	CRTH2 Inhibitor - Oral small molecule	Phase II completed	Novartis	NCT01785602
ILV-094		IL-22	Anti-IL-22 mAb	In Phase II	Pfizer	NCT01941537
GBR830		OX40	Anti-OX40 mAb	In Phase II	Glenmark	NCT02683928
Secukinumab	Cosentyx	IL-17	Anti-IL-17 mAb	In Phase II	Novartis	NCT02594098
OC000459		CRTH2	CRTH2 Inhibitor - Oral small molecule	In phase II	Atopix	NCT02002208
Baricitinib		JAK1/2	Jak1/2 inhibitor - Oral small molecule	In Phase II	Eli Lilly	NCT02576938
PF-04965842		JAK1/2	Jak1/2 inhibitor - Oral small molecule	In Phase II	Pfizer	NCT02780167
ZPL389		H4R	Histamine H4 receptor inhibitor - Oral small molecule	Phase II completed	Ziarco Pharma	NCT02424253
BMS-981164		IL-31	Anti-IL-31 mAb	Phase I completed	BMS	NCT01614756
AMG157/Tezepelumab		TSLP	Anti-TSLP mAb	Phase I completed	Amgen	NCT00757042
MK-8226		TSLPR	Anti-TSLPR mAb	In Phase I	Merck	NCT01732510

CRTH2, Prostaglandin D_2 receptor 2; H4R, histamine H4 receptor; IL-4R, IL-4 receptor; TSLPR, thymic stromal lymphopoietin receptor.
Source: Brunner *et al.* J Allergy Clin Immunol 2017;139:S65-76.

Prevention of AD development

Recent studies have demonstrated that moisturizers reduce rates of AD development[4,104,105] and that probiotic supplementation may prevent AD.[118,119] Additionally, investigators have reported that skin commensal bacteria, including *S. epidermidis* and *S. hominis*, play crucial roles in skin homeostasis and defense against microbial penetration.[47-49] It has also been suggested that colonization by commensal staphylococci can modulate skin immunity and might prevent development of AD.[16,97] Therefore, correcting dysbiosis in AD skin may improve skin barrier function and prevent AD development. Recently, Kelleher *et al.*[120] have demonstrated that increased TEWL at 2 days and 2 months predates and predicts AD at 1 year. Kim *et al.*[121] have also reported that thymic stromal lymphopoietin (TSLP) predicts the development of AD during infancy. These data suggest that detection of increased TEWL, TSLP, and skin dysbiosis in early life might predict AD and facilitate introduction of strategies to prevent AD development. These would include early use of moisturizers, topical anti-inflammatory agents, probiotics as well as correction of microbial dysbiosis.

CONCLUSIONS AND FUTURE DIRECTIONS

Factors, including immune dysregulation, epidermal gene mutations, deficiency of AMPs, and skin dysbiosis, may interact with each other and cause skin barrier defects. Several strategies have been utilized to improve skin barrier function and to control AD. Recently, moisturizers, probiotics, and targeted microbiome therapy have been suggested to prevent AD development in early life. Additionally, broad-spectrum and targeted therapies have been considered to control AD and prevent the atopic march in patients with moderate-to-severe AD. Further studies are warranted to determine the efficacy of these diverse strategies, including emollients, probiotics, and commensal bacteria, to prevent development of AD. It is noteworthy that recent data suggests AD is not just a local skin disease, but a systemic immune disease because nonlesional skin and blood profile show inflammatory findings. Therefore, we may need to expand our scope of management, in the future, to systemic treatment in patients with moderate-to-severe AD.

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