

Barrier Dysfunction Caused by Environmental Proteases in the Pathogenesis of Allergic Diseases

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ABSTRACT

Skin barrier dysfunction has emerged as a critical driving force in the initiation and exacerbation of atopic dermatitis and the “atopic march” in allergic diseases. The genetically determined barrier deficiency and barrier disruption by environmental and endogenous proteases in skin and epithelium are considered to increase the risk of sensitization to allergens and contribute to the exacerbation of allergic diseases. Sources of allergens such as mites, cockroaches, fungi, and pollen, produce or contain proteases, which are frequently themselves allergens. *Staphylococcus aureus*, which heavily colonizes the lesions of atopic dermatitis patients and is known to trigger a worsening of the disease, also produces extracellular proteases. Environmental proteases can cause barrier breakdown in the skin, not only in the epithelium, and stimulate various types of cells through IgE-independent mechanisms. Endogenous protease inhibitors control the functions of environmental and endogenous proteases. In this review, we focus on the barrier dysfunction caused by environmental proteases and roles of endogenous protease inhibitors in the pathogenesis of allergic diseases. Additionally, we examine the subsequent innate response to Th2-skewed adaptive immune reactions.

KEY WORDS

allergen source-derived protease, barrier dysfunction, endogenous protease inhibitor, *Staphylococcus aureus* protease, Th2 adjuvant activity

INTRODUCTION

Allergic diseases are multifactorial, heterogeneous disorders caused by the interaction of environmental and genetic factors. The pathogenesis of allergic diseases has generally been considered to be driven by immunity, for example, IgE overproduction, IgE receptor abnormality, and Th2-skewed Th cell differentiation. Skin barrier dysfunction¹⁻⁴ has emerged as a critical driving force in the initiation and exacerbation of atopic dermatitis (AD) with a recent major breakthrough in the genetics of AD.⁵⁻⁸ Loss-of-function mutations in the gene encoding epidermal structural protein filaggrin (*FLG*) were identified as an important risk factor for AD.⁵ The *FLG* mutations are associated with more severe AD, early onset of AD, enhanced

systemic sensitization, and, interestingly, increased risk for asthma in patients with a previous history of eczema,⁵⁻⁷ i.e. features of the “atopic march”.^{9,10} Asthma can also be considered a disease involving an impaired barrier function of the airway epithelium.^{11,12}

An understanding of the mechanism of primary sensitization to allergens is important in elucidating the pathogenesis of allergic diseases and for possibly preventing their development. Sources of allergens, such as mites, cockroaches, fungi, and pollen, produce or contain proteases.¹³⁻¹⁹ Proteolytic activity has been proposed to be involved in the pathogenesis of allergies by facilitating the passage of allergens across tissue barriers, cleaving various molecules, and affecting the functions of various cells and im-

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immune responses.^{13-16,20,21} The proteases involved are frequently themselves allergens, recognized by IgE in sensitized patients. For example, house dust mites produce at least four groups of protease allergens.²² *Staphylococcus aureus*, which heavily colonizes the lesions of AD patients and is known to trigger a worsening of the disease, also produces extracellular proteases.²³

Environmental proteases have been demonstrated to cause barrier breakdown even in skin,²⁴⁻²⁷ not only in the epithelium,²⁸⁻³² providing a portal of entry for allergens, irritants, and molecules derived from allergen sources and microbes that initiate and perpetuate inflammation. Environmental proteases also stimulate various types of cells through IgE-independent mechanisms.^{13-16,20,21,33-48} These findings imply the importance of environmental proteases as a factor contributing to primary sensitization to allergens and the exacerbation of allergic diseases by abrogating the epidermal/epithelial barrier and by inducing innate responses. The genetically determined barrier deficiency and the barrier disruption by environmental and endogenous proteases are considered to increase the risk of sensitization to allergens leading to IgE production and contribute to the exacerbation of allergic diseases. Endogenous protease inhibitors control the activity of environmental and endogenous proteases.^{37-39,49-51} In this review, we focus on the barrier dysfunction caused by environmental proteases and roles of endogenous protease inhibitors in the pathogenesis of allergic diseases. We also examine the subsequent innate immune response to Th2-skewed adaptive immune reactions (Fig. 1).

ALLERGEN SOURCE-DERIVED PROTEASES

Sources of allergens, such as mites, cockroaches, fungi, and pollen, produce or contain proteases.¹³⁻¹⁹ The proteases themselves are frequently allergens, recognized by IgE in sensitized patients. For example, house dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, produce at least four groups of protease allergens, major group 1 allergens with cysteine protease activity and group 3, 6, and 9 allergens with serine protease activity.²² Some preparations of natural mite group 1 allergens, Der p 1 and Der f 1, exhibit not only cysteine protease activity but also serine protease activity.⁵²⁻⁵⁵ However, it is clear that the group 1 allergens only possess cysteine protease activity and the serine protease activity detected within some preparations of natural mite group 1 allergens is due to contamination by mite-derived serine protease(s).⁵⁵ Considerable cysteine protease activity of the mite group 1 allergens is detected in mite body and fecal extracts.^{56,57} In contrast, serine protease activity is much more abundant in the fecal than body extract.^{58,59}

Grains of allergenic pollen release proteases.^{18,19} At least one molecular species of serine protease was

released into phosphate-buffered saline from the pollen grains of Japanese cedar, Japanese cypress, and Rocky mountain juniper, which belong to the *Cupressaceae/Taxodiaceae* family, and birch, short ragweed, and two grasses, Kentucky blue and cultivated rye, at physiological conditions without sonication.¹⁹ In the *Cupressaceae/Taxodiaceae* family, sonication was necessary for the release of another serine protease.^{18,19} In birch, ragweed, and the grasses, most of the serine and cysteine proteases were released without sonication.^{18,19} The released proteases digested gelatin and/or casein differently among plant species.¹⁹ Two serine proteases from short ragweed pollen have been purified and characterized.^{60,61} An aminopeptidase from Japanese cedar pollen has been purified and characterized^{62,63} and, very recently, Ibrahim *et al.*⁶⁴ reported that a novel Japanese cedar pollen allergen identified by a proteomic approach is an aspartic protease. Grobe *et al.*^{65,66} have proposed that grass pollen major group 1 allergens are cysteine proteases and Raftery *et al.*⁶⁷ identified a protease derived from Bermuda grass pollen as Cyn d 1, the Bermuda grass pollen group 1 allergen, by a proteomic approach. However, there is a conflicting report.⁶⁸

DISRUPTION OF EPITHELIAL BARRIER AND CLEAVAGE OF TIGHT JUNCTION PROTEINS BY ALLERGEN SOURCE-DERIVED PROTEASES

Robinson and coworkers²⁸ suggested that cysteine protease activity of Der p 1 causes the bronchial epithelium to become increasingly permeable to macromolecules as a consequence of proteolytic injury by demonstrating that Der p 1 elicits detachment of the Madin-Darby canine kidney (MDCK) cell line and primary cultures of canine tracheal epithelium from artificial and matrix protein substrata, that Der p 1 causes an increase in the permeability of the bronchial epithelium to albumin, and that Der p 1 causes histologically observable damage to the architecture of the epithelium in bovine bronchial segments.

They also found that cysteine protease activity of Der p 1²⁹ and serine protease activity from mite fecal pellets³⁰ cause the disruption of intercellular tight junctions (TJs), which are the principal components of the epithelial paracellular permeability barrier,^{11,12,69,70} and increased epithelial permeability in MDCK, 16HBE14o-, or Calu-3 cells, the latter two of which are immortalized cell lines derived from human bronchial epithelium. Der p 1 and mite-derived serine protease activity cleaved occludin, a TJ adhesion protein, and caused intracellular proteolysis of ZO-1, a cytoplasmically localized TJ protein. Putative cleavage sites were found in peptides from an extracellular domain of occludin and claudin-1, another TJ adhesion protein.

Pollen extracts of Kentucky blue grass, birch, giant ragweed, and Easter lily also degraded TJs in MDCK

Barrier Dysfunction Caused by Proteases

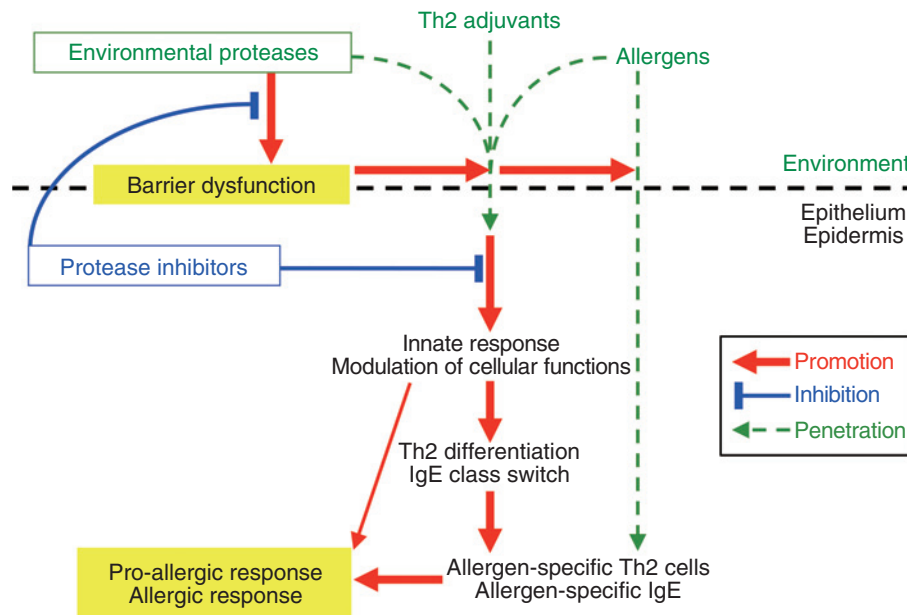


Fig. 1 Roles of environmental proteases and endogenous protease inhibitors in allergic diseases. Environmental proteases cause a breakdown of the epithelial barrier and more rigid skin barrier, providing a portal of entry for themselves, allergens, microbial products, and irritants; and stimulate directly or indirectly various types of cells through IgE-independent mechanisms, inducing proinflammatory and proallergic responses and leading to Th2-skewed adaptive immune responses. Environmental proteases, components of allergen sources, and microbial products exhibit Th2-adjuvant activity. Some allergen proteins (protease allergens and non-protease allergens) exhibit Th2-adjuvant activity.

or Calu-3 cells and pollen extract of Kentucky blue grass did so in a manner dependent on serine and cysteine protease activities in MDCK cells.³¹ Pen ch 13, a major serine protease allergen of airborne fungi, has also been reported to cleave occludin in 16HBE14o- cells.³²

These reports suggest that the allergen source-derived proteases, which degrade TJs in epithelium and epidermis,^{11,12,69,70} increase the accessibility of both themselves and other allergens to dendritic antigen-presenting cells or other types of cells, such as mast cells and basophils, which can respond to proteases in a manner independent of IgE or to allergens via the crosslinking of complexes of high affinity IgE receptors and allergen-specific IgE on their cell-surface.

EPIDERMAL BARRIER DYSFUNCTION CAUSED BY ALLERGEN SOURCE-DERIVED PROTEASES

INDUCTION OF BARRIER BREAKDOWN BY A MITE PROTEASE

Skin barrier dysfunction¹⁻⁸ has emerged as a critical driving force in the initiation and exacerbation of AD and the “atopic march”^{9,10} in allergic diseases. The critical permeability function of the skin is mediated by the outermost layer of the epidermis, the stratum

corneum (SC).⁷¹⁻⁷⁴ Although allergen source-derived proteases have been suggested to disrupt the bronchial epithelial barrier,²⁸⁻³² the skin barrier is considered a much more rigid system. We reported the first demonstration of the barrier function of the skin being disrupted by allergen-derived proteolytic activity *in vivo*.²⁴ We examined whether the cysteine protease activity of a recombinant form of Der f 1 causes a reduction in the barrier function of the skin in nude mice with the BALB/c background. The use of nude mice simplified the experimental procedures because of the hairless phenotype, and effects of T-cell-mediated acquired immunity on the barrier dysfunction could be ignored because the mice lack a thymus. Dry skin and an impaired barrier function are hallmarks of AD. The barrier function was evaluated based on two parameters, transepidermal water loss (TEWL) and the penetration by riboflavin of the SC. Application of the recombinant Der f 1 with occlusion to the backs of nude mice for one week induced increases in TEWL and the penetration by riboflavin of the SC at the treated sites and morphological changes to the skin’s surface when the proteolytic activity was induced, but not when it was inhibited by an irreversible cysteine protease-specific inhibitor, E-64.

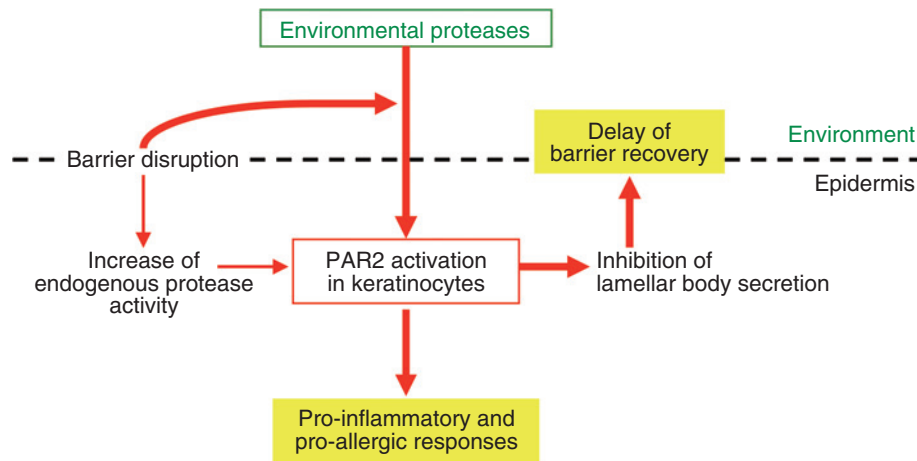


Fig. 2 Roles of epidermal PAR2 activation by environmental proteases. The activation of PAR2 by environmental proteases induces proinflammatory and proallergic responses in keratinocytes (and also epithelial cells). In barrier-disrupted skin, the activation of PAR2 in keratinocytes inhibits the secretion of lamellar bodies critical to maintenance of the permeability barrier's homeostasis and delaying barrier recovery.

ENVIRONMENTAL PROTEASE-INDUCED DELAY OF BARRIER RECOVERY IN BARRIER-DISRUPTED EPIDERMIS

Acute disruption of the permeability barrier by repeated tape stripping increased endogenous serine protease activity and the topical application of serine protease inhibitors accelerated the barrier's recovery.^{75,76} The lamellar body (LB) secretory system is critical for maintenance of the permeability barrier's homeostasis.⁷⁷ Hachem *et al.*⁷⁶ demonstrated that tape stripping-induced barrier disruption provoked the activation of the protease-activated receptor (PAR)-2 (PAR2)^{78,79} and topical application of the PAR2 agonist peptide, SLIGRL, inhibited LB secretion and delayed recovery, while PAR2 knockout mice displayed an accelerated barrier recovery and enhanced LB secretion, identifying PAR2 as a novel signaling mechanism of epidermal permeability barrier homeostasis.

Jeong *et al.*²⁵ recently reported that cockroach and mite extracts delayed epidermal permeability barrier recovery (evaluated by measuring TEWL) when topically applied to barrier-disrupted sites (by repeated tape stripping) on mouse (hairless mice) and human skin and that, at least for the cockroach extract, serine protease activity was responsible for the effect. In mice, topical application of the cockroach extract to barrier-disrupted skin caused a decrease of neutral lipid deposition in the SC and delay of LB secretion along with the delayed barrier recovery, and topical application of the PAR2-specific antagonist ENMD-1068 or a protease inhibitor normalized the barrier recovery. The cockroach extract they used activated primary human keratinocytes via PAR2.²⁵ Kato *et al.*³⁶ demonstrated that mite-derived serine protease activ-

ity activated primary human keratinocytes via PAR2.

Mite allergens are an important factor in AD⁸⁰⁻⁸² and the mite group 1 allergens, Der f 1 and Der p 1, are present on human skin.⁸³ Fecal pellets of *D. pteronyssinus* are known to contain Der p 1 at high concentrations (10 mg/ml)⁸⁴ together with other serine proteases.²² Areas of the skin where the barrier function is reduced are likely to provide a portal of entry for allergens and irritants. Results by Nakamura *et al.*²⁴ suggested that the proteolytic activity of mite major group 1 allergens reduces the barrier function of skin in the initiation of allergen-sensitization through the skin (Fig. 1), while those by Jeong *et al.*²⁵ suggested that PAR2's activation by proteases derived from sources of allergens such as cockroaches²⁵ and mites³⁶ delays LB secretion critical for maintenance of permeability barrier homeostasis and subsequent recovery of the epidermis (Fig. 2), promoting allergen-sensitization through the barrier-disrupted skin or exacerbating AD.

ALLERGIC RISK POSED BY A GENETICALLY DETERMINED BARRIER DEFICIENCY AND ENVIRONMENTAL PROTEASES

The combination of a genetically determined barrier deficiency and the promotion of barrier disruption by environmental proteases might increase the risk of sensitization to allergens through the skin leading to the production of IgE and exacerbation of AD. Interestingly, Moniaga *et al.*⁸⁵ very recently reported that a mite extract-induced dermatitis physiologically relevant to human AD was enhanced in Flaky tail (*Flg^{fl}*) mice, essentially deficient in filaggrin, compared to C57BL/6 mice, although whether mite-derived protease activity or other factors contribute to the model

is yet unknown.

EPIDERMAL BARRIER DYSFUNCTION CAUSED BY A STAPHYLOCOCCUS AUREUS EXTRACELLULAR PROTEASE

STAPHYLOCOCCUS AUREUS AND AD

S. aureus heavily colonizes the lesions of AD patients. *S. aureus* is not a normal member of the microflora colonizing the skin. In AD patients, however, *S. aureus* colonizes lesional skin and colony counts even in nonlesional skin are often high.⁸⁶ Deficiency in the function or expression of antimicrobial peptides in skin and in the Th2 cytokine milieu accounts for the susceptibility of AD patients to *S. aureus*.^{87,88} The severity of AD is reduced by treatment with a combination of antistaphylococcal antibiotics and topical glucocorticoid,^{89,90} suggesting an important contribution of *S. aureus* to the pathogenesis of AD.

INDUCTION OF BARRIER BREAKDOWN BY A S. AUREUS PROTEASE

S. aureus is known to produce extracellular proteases, including serine proteases, cysteine proteases, and metalloproteases.²³ Very recently, we examined whether a *S. aureus* extracellular serine protease, commonly referred to as V8 protease, impairs the epidermal permeability barrier in mice.²⁷ The protease's application with occlusion to the backs of nude mice for one week induced permeability barrier dysfunction, which was evaluated based on TEWL and the penetration by riboflavin of the SC. We also evaluated effects of V8 protease with or without occlusion in hairless mice, which show the hairless phenotype but are normal in the development of the immune system. Application of the protease with or without occlusion increased the TEWL in a time-dependent manner. Analysis by transmission electron microscopy revealed that the protease induced structural disturbances in the SC. Histology revealed that the protease without occlusion induced an increase in epidermal thickness and, with occlusion, induced an even greater increase in epidermal thickness and inflammatory infiltration in the dermis.

Interestingly, exfoliative toxins (ETs) from *S. aureus* show sequence similarity to V8 protease and exhibit a similar glutamate-specific cleavage to V8 protease.²³ ETs cause blisters in bullous impetigo and staphylococcal scaled-skin syndrome, and hydrolyze a single peptide bond after glutamate 381 of desmoglein 1 (Dsg1), a desmosomal cadherin that mediates cell-cell adhesion.^{91,92} Considering their similarity in structure and substrate specificity, the staphylococcal glutamate-specific protease family including ETs and V8 protease may abrogate the epidermal barrier via Dsg1 cleavage in the corneodesmosomes which causes the loss of corneocyte cohesion and structural disturbance of the SC. On the other hand, the highly specific nature of the cleavage after glutamate sug-

gests that the family may not activate PAR2 directly because the cleavage site for PAR2 activation is a peptide bond after arginine,⁷⁹ although there may be a cascade in the activation of proteases and the family may activate PAR2 secondarily.

The breakdown of the epidermal permeability barrier and structural disturbance of the SC caused by the *S. aureus* extracellular protease suggest that the residential *S. aureus* could impair the epidermal barrier. Areas of the skin where the barrier function is impaired are likely to provide a portal of entry for various *S. aureus*-derived molecules,^{93,94} allergens such as house dust and pollen, and irritants that all initiate and perpetuate cutaneous inflammation. The results by Hirasawa *et al.*²⁷ imply the importance of *S. aureus* proteases as an environmental factor, which contributes to primary sensitization to allergens, and the vicious cycle of *S. aureus* colonization and exacerbation of AD by abrogating the epidermal permeability barrier.

ENVIRONMENTAL PROTEASES AS Th2 ADJUVANTS

Proteolytic activity has been proposed to be involved in the pathogenesis of allergies via not only disruption of epithelial/epidermis barriers as described above but also cleavage of endogenous protease inhibitors, induction of proinflammatory and proallergic responses, and promotion of Th2 skewing and IgE production.^{13-16,20,21,27,33-48} These imply the importance of environmental proteases as an environmental factor that contributes to primary sensitization to allergens and exacerbation of allergic diseases by not only abrogating the epidermal/epithelial barrier but also inducing an innate response towards Th2-skewed adaptive immune responses (Fig. 1).

PROTEASE-DEPENDENT ACTIVATION OF EPITHELIAL CELLS AND KERATINOCYTES

The epidermis and epithelium are located at the interface between the body and environment and have critical roles in the response to various stimuli during the sensitization and effector phases in allergic diseases.^{12,20,21,95} Mite-derived protease activities have been reported to induce the release of proinflammatory cytokines such as IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), eotaxin, and/or IL-6 in airway³³⁻³⁵ and conjunctival³⁹ epithelial cells and keratinocytes.³⁶⁻³⁸ Mite-derived serine protease activity induces proinflammatory responses in airway epithelial cells and keratinocytes via PAR2's activation (Fig. 2) but the cysteine protease activity of mite group 1 allergens acts via another unknown mechanism.^{33,36} Cytokines and chemokines induced by stimulation with mite proteases in the epithelium/epidermis may attract and/or activate inflammatory cells and antigen-presenting cells. Along with the proinflammatory cytokine and chemokine responses, in

barrier-disrupted epidermis, activation of PAR2 by allergen source-derived proteases inhibits LB secretion to delay barrier recovery^{25,26} (Fig. 2).

Trypsin (serine protease), papain (cysteine protease), and an extract of airborne *Alternaria* have recently been reported to induce the release of thymic stromal lymphopoietin (TSLP), which plays a key role in allergic diseases,⁹⁶ in the human bronchial epithelial cell line BEAS-2B.⁹⁷ Upregulation of TSLP gene expression was inhibited by transfection with PAR2 siRNA completely for trypsin and partially for papain and the *Alternaria* extract.

PROTEASE-DEPENDENT ACTIVATION OF INFLAMMATORY CELLS AND AIRWAY SMOOTH MUSCLE

Furthermore, mite-derived protease activity could activate inflammatory cells,⁴¹⁻⁴⁴ which might be attracted to the mite protease-exposed epithelium/epidermis, and airway smooth muscle.⁴⁰ IgE-independent activation of mast cells⁴¹ and basophils^{41,42} has been reported. The cysteine protease activity of Der f 1 and papain, homologous to mite group 1 allergens, induces the degranulation of eosinophils,⁴³ and Fujisawa *et al.*⁴⁴ reported that mite extract induced eosinophils to express IL-9 and this was inhibited by a serine protease-specific inhibitor.

Interestingly, the cysteine protease activity of highly purified recombinant mite group 1 allergens was crucial to the sensitization process in mice,⁴⁶ and Sokol *et al.*⁴⁷ demonstrated that the cysteine protease activity of papain initiates Th2 sensitization in mice via the activation of basophils to produce Th2-inducing cytokines including IL-4. Very recently, Tang *et al.*⁴⁸ suggested that the Th2-inducing adjuvant activity of papain occurred through cooperation between dendritic cells, which are conditioned by TSLP induced in the epidermis, and basophils.

OTHER ENVIRONMENTAL Th2 ADJUVANTS

The barrier defect induced by environmental proteases such as those from allergen sources and *S. aureus* increase the accessibility of not only the proteases themselves but also other allergens and molecules produced by or contained in allergen sources, microbes, and house dust (Fig. 1). Some of them have been suggested to have Th2-adjuvant activity.^{19,93,94,98-107} These components could act synergistically to form a microenvironment in which physical and biochemical barriers are disrupted, inflammatory cells are attracted and activated, and functions of antigen-presenting cells are modified to exhibit Th2-polarizing activity. Such a microenvironment would be optimal for the initiation of allergic sensitization and/or exacerbation of allergic inflammation.

ENDOGENOUS PROTEASE INHIBITORS

CYSTATIN A

Kato *et al.*³⁷ found that human sweat inhibited the proteolytic activities of recombinant forms of mite group 1 allergens, Der f 1 and Der p 1, and used sweat as a skin-derived material for isolation of the dominant inhibitor, which was subsequently determined to be cystatin A (also known as stefin A^{108,109}). Moreover, stimulation of keratinocytes with the cysteine protease activity of these allergens promoted the secretion of IL-8 and GM-CSF, and the addition of cystatin A blocked this.^{37,38} Thus, a system including the activation and suppression of keratinocytes in response to mite cysteine proteases exists in the skin (Fig. 1). Cystatin A was stable even though it interacted with active recombinant Der p 1 or Der f 1,³⁸ and this could be explained by the high-affinity binding of this inhibitor to the cysteine protease and that the N-terminal hook of cystatin family inhibitors binds to part of the substrate binding cleft of cysteine proteases in a substrate-like manner but then turns away from the active site, preventing proteolytic processing.¹¹⁰ The stability of cystatin A is considered to contribute to a homeostatic role against skin inflammation.

Indeed, cystatin A content is reported to have decreased in the lesional skin of patients with AD,¹¹¹ and, recently, Vasilopoulos *et al.*¹¹² found a significant association of a cystatin A genotype, in which mRNA of cystatin A is unstable, with AD. Thus, decrease of cystatin A by non-genetic¹¹¹ or genetic¹¹² factors could increase the risk for sensitization through the skin and the perpetuation of AD. Protease inhibitors such as cystatin A could function as a biochemical skin barrier^{37,38} to prevent the activation of keratinocytes or other types of cells by environmental proteases causing Th2 induction and IgE production. A suppressive effect of cystatin A on induction of Der f 1-specific IgE in the sensitization process has recently been demonstrated in mice, *i.e.* the first example that an endogenous protease inhibitor attenuated *in vivo* IgE-inducing immunogenicity of a cysteine protease-allergen.¹¹³ Interestingly, Takahashi *et al.*^{114,115} reported that phosphorylated rat cystatin α (a rat counterpart of cystatin A) inhibited the activity of a *S. aureus*-derived cysteine protease and poliovirus 3C protease and suppressed *S. aureus* growth on the skin of newborn rats and replication of the poliovirus in HeLa cells. Analysis of interactions between environmental and genetic factors such as those between environmental proteases and their endogenous inhibitors is important to elucidate the pathogenesis of allergic diseases and to prevent their development.

SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA2)

Sakata *et al.*^{49,50} reported that SCCA2 inhibits the cys-

teine protease activity of Der p 1 and Der f 1. Th2 cytokines (IL-4 and IL-13) induce the expression of SCCA2 in bronchial epithelial cells and keratinocytes and SCCA2 expression is augmented in asthma patients and skin lesions of AD patients.^{116,117} SCCA inducible in the Th2 cytokine milieu-conditioned epithelium and epidermis may provide a negative feedback to regulate excessive promotion of Th2 induction by the cysteine protease activity of the allergens.⁴⁹

ELASTASE INHIBITORS

Endogenous protease inhibitors, secretory leukocyte protease inhibitor (SLPI) and α 1-antitrypsin, inhibited the mite-derived serine protease-dependent release of IL-8 and IL-6 in conjunctival epithelial cells.³⁹

α 1-antitrypsin, elafin (also known as skin-derived antileukoprotease/SKALP/trappin-2), and SLPI are known as elastase inhibitors, which inhibit the protease activity of neutrophil elastase and key molecules in the defense of the lung. Brown *et al.*⁵⁴ reported that mite-derived cysteine and serine proteases disturbed innate defense mechanisms of the lung by cleaving and inactivating some of the elastase inhibitors. Cysteine protease activity of Der p 1 cleaved endogenous protease inhibitors, human α 1-antitrypsin,^{54,118,119} human elafin, and murine SLPI but not human SLPI.⁵⁴ Mite-derived serine protease activity, which contaminated a natural Der p 1 preparation, cleaved human elafin but not human α 1-antitrypsin and human and murine SLPI.⁵⁴

LYMPHOEPITHELIAL KAZAL-TYPE RELATED INHIBITOR (LEKTI)

Netherton syndrome is a severe genetic skin disease with constant atopic manifestations that is caused by mutations in *SPINK5* (serine protease inhibitor Kazal-type 5), which encodes LEKTI.^{120,121} A lack of LEKTI causes SC detachment secondary to epidermal protease hyperactivity.¹²² LEKTI inhibits the protease activity of epidermal kallikrein (KLK) 5, KLK7, and KLK14.⁵¹ KLK5 directly activates PAR2 to upregulate the gene expression of TSLP, ICAM1, IL-8, and TNF- α in keratinocytes.¹²³ At embryonic day 19.5, *Spink5/Par2* double knockout mice display a dramatic decrease in TSLP expression, confirming the role of the KLK5-PAR2 cascade in TSLP-mediated early proallergic signaling.¹²⁴ However, deletion of *Par2* in adult double knockout-grafted skin does not rescue the inflammatory phenotype. The results¹²⁴ suggest that several mechanisms (skin barrier dysfunction, mechanical stress induced by SC detachment, as well as protease-induced pathways including KLK5-PAR2-mediated overexpression of TSLP¹²³ and the consequence of other hyper activated proteases^{122,125,126}) trigger and maintain the inflammatory phenotype in Netherton syndrome. Whether LEKTI inhibits the activity of environmental pro-

teases is unknown.

CONCLUSIONS

Environmental proteases (allergen source-derived proteases and microbial proteases) cause a breakdown of not only the epithelial barrier but also the more rigid skin barrier, providing a portal of entry for themselves, allergens, microbial products, and irritants; and directly or indirectly stimulate (or modulate functions of) various types of cells (epithelial cells, keratinocytes, basophils, mast cells, eosinophils, airway smooth muscle, antigen-presenting cells, and other types of cells) through IgE-independent mechanisms, inducing proinflammatory and proallergic responses and leading to Th2-skewed adaptive immune responses. Direct or indirect targets of environmental proteases include epithelial and epidermal TJs, molecules essential to structural integrity of the SC such as Dsg1, endogenous protease inhibitors and molecules protective of tissues, PAR2, and others. Endogenous protease inhibitors control the functions of environmental and endogenous proteases. Analysis of the interactions between environmental and genetic factors including environmental proteases, their endogenous inhibitors, protease-sensing pathways, and innate immunity is important to elucidate the pathogenesis of allergic diseases and to prevent their development.

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