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The role of protease activation of inflammation in allergic respiratory diseases

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Extracellular endogenous proteases, as well as exogenous proteases from mites and molds, react with cell-surface receptors in the airways to generate leukocyte infiltration and to amplify the response to allergens. Stimulation leads to increased intracellular Ca^{++} and gene transcription. The most thoroughly investigated receptors, protease-activated receptors (PARs), are 7-transmembrane proteins coupled to G proteins. PARs are widely distributed on the cells of the airways, where they contribute to the inflammation characteristic of allergic diseases. PAR stimulation of epithelial cells opens tight junctions, causes desquamation, and produces cytokines, chemokines, and growth factors. They degranulate eosinophils and mast cells. Proteases contract bronchial smooth muscle and cause it to proliferate. PARs also promote maturation, proliferation, and collagen production of fibroblast precursors and mature fibroblasts. PAR-2, apparently the most important of the 4 PARs that have been characterized, is increased on the epithelium of patients with asthma. Trypsin, a product of injured epithelial cells, and mast cell tryptase are potent activators of PAR-2. Mast cell chymase activates PAR-1. Proteases from mites and molds appear to act through similar receptors. They amplify IgE production to allergens, degranulate eosinophils, and can generate inflammation, even in the absence of IgE. Proteases produced by *Aspergillus* species to support its growth are presumably responsible for the exuberant IgE, IgG, and granulomatous response of allergic bronchopulmonary aspergillosis. Similar proteases from molds germinating on the respiratory mucosa have been recently been implicated in the pathogenesis of chronic hyperplastic rhinitis and polyps and, by extension, of intrinsic asthma. Finally, proteases from mites and fungi growing in damp, water-damaged buildings might be the basis for the increased prevalence in these buildings of rhinitis, asthma, and other respiratory diseases. Future research promises to promote our understanding of the pathogenesis of allergic respiratory diseases and point the way to new therapies.
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Despite the impressive advances in our knowledge of the cell biology of allergic diseases, there remain a great many unanswered questions. The effects of stimulation by proteases acting through membrane receptors answer some of these questions and offer promise of answers to many more.

Protease-activated receptors (PARs) were first identified as a mechanism for the interaction between blood clotting and platelet activation. Initially, they were investigated for their role in injury and wound healing; more recently, their role in inflammation has become a focus of attention.[1-6] PARs are 7-transmembrane G protein–coupled receptors stimulated by serine proteases (Fig 1). Four PARs have been identified and cloned. They are widely expressed on cells in blood vessels, connective tissue, leukocytes, epithelium, and many airway cells.[6] Serine proteases cleave the amino acids at a specific site of the extracellular N-terminus of the molecule to expose a new N-terminal ligand domain that binds to another site on the same molecule, thereby activating the receptor. The amino acid sequence of each cleavage site is specific for the particular PAR, and mAb assays for the protein and PCR assays for mRNA are available. Synthesis of specific peptide agonists and antagonists has been an essential step in elucidation of the actions of PARs.

The proteolytic activation is irreversible, and once cleaved, the receptors are degraded in lysosomes. Thrombin activates PAR-1, PAR-3, and PAR-4; trypsin activates PAR-2 and PAR-4; and of particular interest for its role in allergic diseases, mast cell tryptase can also activate PAR-2. Activated PARs couple to G-signaling cascades that increase phospholipase C levels, which in turn leads to increased intracellular Ca^{++} levels.[7-9] G protein activation also generates transcriptional responses through extracellular signal–regulated kinase and mitogen-activated protein kinase and nuclear factor κB.[10-12] The details of intracellular signal transduction cascades

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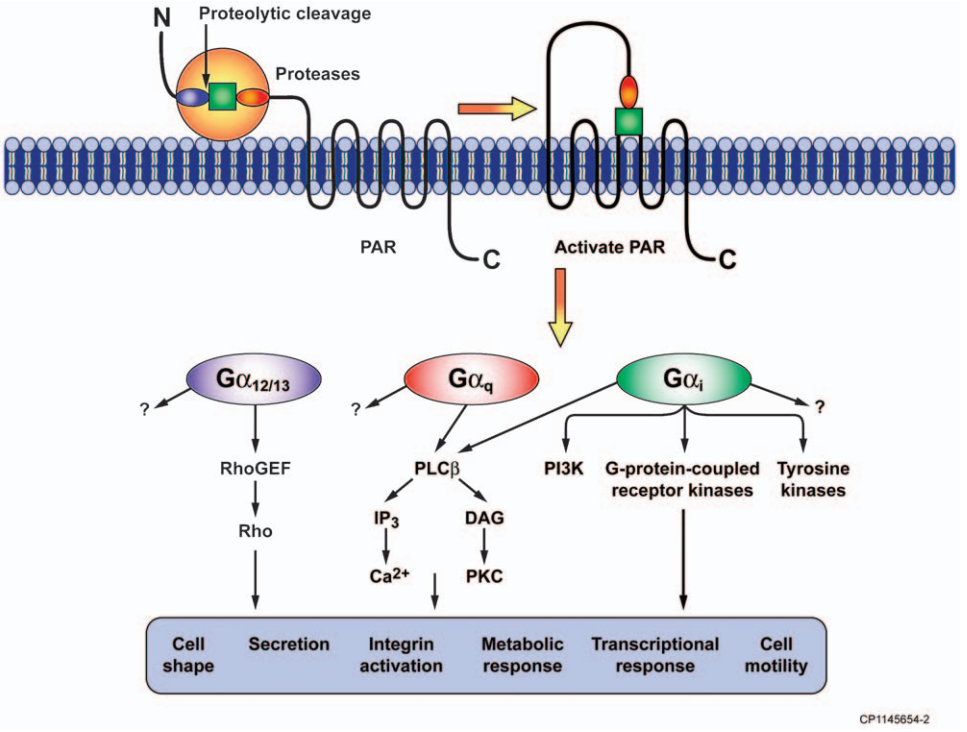

FIG 1. Signal transduction pathways of the PAR response. *RhoGEF,* Rho guanine nucleotide exchange factor, *PLCβ,* phospholipase Cβ; *PI3K,* phosphoinositide 3-kinase; *IP_3,* inositol triphosphate; *DAG,* diacylglycerol; *PKC,* protein kinase C.

Abbreviations used
PAR: Protease-receptor
PDGF: Platelet-derived growth factor
PGD_2: Prostaglandin D_2

are complex. Coupling to specific G proteins probably varies between different PARs and between different cells, and the response doubtless depends on interaction with other signal cascades stimulated by agents like cytokines, chemokines, or neurotransmitters. The effects of increased intracellular Ca^{++} include secretion, degranulation, and smooth muscle contraction. Gene transcription produces integrins, chemokines, and cytokines, as well as cyclooxygenase 2. PARs cause edema, promote angiogenesis and fibrosis, and enhance IgE production, leukocyte infiltration, and airway hyperresponsiveness.

In addition to endogenous extracellular proteases, some exogenous serine and cysteine proteases from molds and mites activate similar signal transduction cascades, but the details of these pathways are not fully defined.[13,14]

Although investigation of the role of protease agonists in allergic diseases is just beginning, it is apparent that these responses can be considered an important form of innate immunity. Their involvement in allergic inflammation and airway remodeling offers promise of explaining many things that have thus far been poorly understood. The aim of this review is to summarize the present state of knowledge and indicate possible future developments.

FUNCTION OF PROTEASES ON CELLS INVOLVED IN ALLERGIC RESPIRATORY DISEASES

PARs are present on virtually all of the cells involved in rhinitis and asthma: epithelium, mast cells, eosinophils, neutrophils, monocytes-macrophages, lymphocytes, smooth muscle, endothelium, fibroblasts, and neurons (Fig 2 and Table I).[2,3,5,7,13,15-65]

Patients with asthma express an increased amount of PAR-2 on respiratory epithelial cells but not on smooth muscle or alveolar macrophages.[15,16] Schmidlin and colleagues[17] have studied the effect of PAR-2 on ovalbumin challenge of immunized mice. Compared with wild-type animals, eosinophil infiltration was inhibited by 73% in mice lacking PAR-2 and increased by 88% in mice overexpressing PAR-2. Similarly, compared with wild-type animals, airway hyperreactivity to inhaled methacholine was diminished by 38% in mice lacking PAR-2 and increased by 52% in mice overexpressing PAR-2. PAR-2 deletion also reduced IgE levels to ovalbumin sensitization 4-fold compared with levels seen in wild-type animals. Mast cell chymase induces eosinophil infiltration, presumably by activating PAR-1.[18] Secretory leukocyte

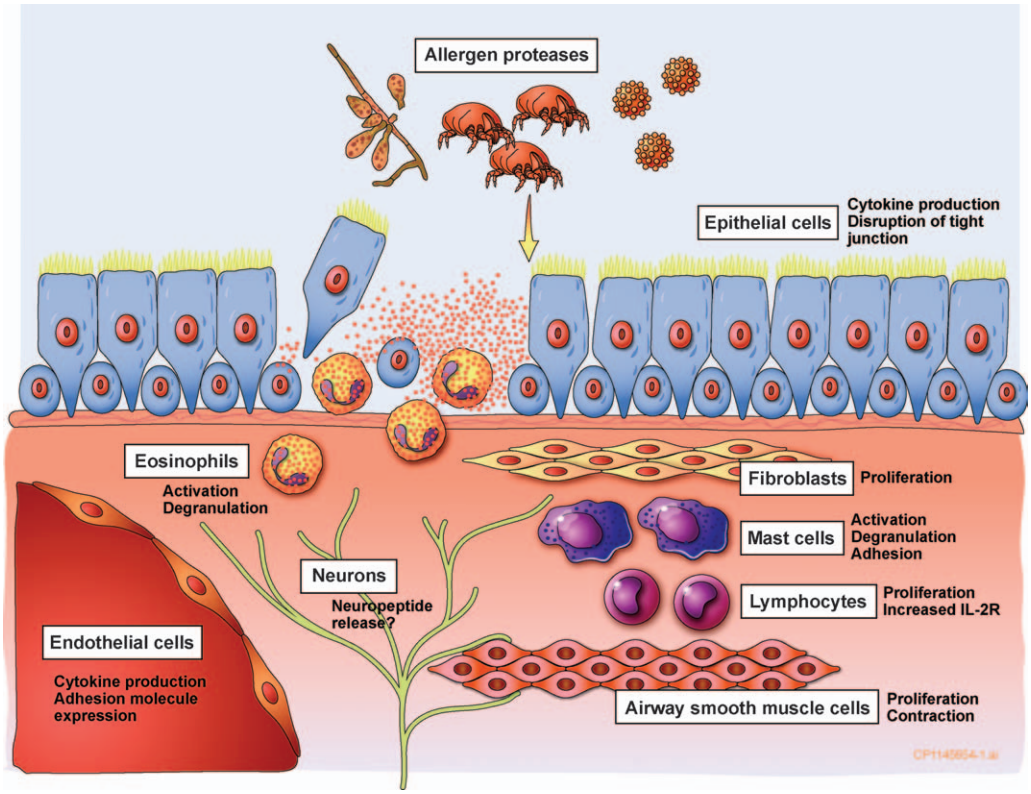

FIG 2. PAR inflammation of the airways: cellular responses.

protease inhibitor administered intratracheally before allergen challenge prevented bronchoconstriction, airway hyperresponsiveness, and leukocyte influx.[66] Stimulation by the protease Der p 1 increases total IgE and specific IgE levels to an unrelated allergen administered at the same time.[19,20]

Murine mast cells express PAR-1 and PAR-2, and therefore presumably stimulation of these receptors can lead to mast cell degranulation.[21,22] However, in a rat peritoneal mast cell preparation, trypsin and tryptase stimulation of PAR-2 failed to induce β-hexosaminidase release, but PAR-activating peptides did.[21] In another study thrombin did stimulate heparin and hexosaminidase release.[23] In a murine mast cell preparation thrombin stimulated IL-6 and matrix metalloproteinase 9 release and mast cell adherence to fibronectin.[24] Also, thrombin enhanced mast cell response to low-level stimulation of FcεRI.[25] These results suggest that murine mast cell PAR-1, but not PAR-2, responds to stimulation. More information is needed about human mast cells: Do they respond by releasing granules? Do mucosal and connective tissue mast cells differ, and do human mast cells in different sites respond differently? An especially important area of interest is their response to tryptase and chymase released from other mast cells as a possible explanation for the apparent feedback amplification of anaphylaxis, especially idiopathic anaphylaxis. It is also important to determine the role of tryptase in basophils and whether PAR stimulation causes basophil granule release.

Eosinophils express PAR-2 and PAR-3.[26] Stimulation of PAR-2 results in superoxide production and degranulation. Eosinophils respond similarly to the cysteine proteases papain and Der f 1, presumably through an as-yet-uncharacterized protease receptor.[13] Response to cysteine proteases was enhanced by IL-5. PAR stimulation by chymase augments neutrophil and eosinophil recruitment.[18] PAR agonists increase endothelial cell expression of E- and P-selectins and intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, as well as production of the chemokines IL-8 and monocyte chemotactic protein 1.[18,27-29]

In animal models and *in vitro* studies of isolated cells, PAR-2 stimulation increases smooth muscle intracellular Ca^{++} with resulting bronchial contraction,[7,30] and the response to histamine and methacholine is increased.[17,31] The cyclooxygenase inhibitor indomethacin increases the contractile response.[31,32]

Airway epithelium expresses PAR-1, PAR-2, and PAR-4. The result of stimulation of these receptors is similar, but response to PAR-2 is much greater. It is of interest that epithelial cells themselves are an important source of trypsin, an agonist for PAR-2.[33] Protease stimulation of airway epithelium produces the cytokines IL-6 and GM-CSF, the chemokines IL-8 and eotaxin, and the platelet-derived growth factor (PDGF).[34-37] Intracellular Ca^{++} is increased, and cyclooxygenase activation generates prostaglandin D_2 (PGD_2).[35,38,39] Interestingly, stimulation of epithelial cell PARs causes bronchial relaxation,[33] but

TABLE I. Effects of PAR stimulation

Cell	PAR-1	PAR-2	PAR-3	PAR-4	Cysteine protease
Airway epithelium	Production of IL-6, IL-8, PGE_2, but less than PAR-2[35]; production of PDGF[34]	Expression increased in asthma[15]; Ca^{++} increase[38]; increased IgE production and increased methacholine response[17]; production of IL-6 and IL-8[33,35-37]; production of GM-CSF and eotaxin[39,52]; production of COX-2 and PGD_2 with relaxation of bronchus[33,35]; production of metalloproteinase 9[53]		Production of IL-6, IL-8, PGE_2, but less than PAR-2[35]	Disruption of tight junctions[54]
Mast cells	Present[21,22]; adhesion to fibronectin[24]; activation[21]; degranulation[23]; IL-6 production[24,25]; augments response to FcεRI[25]; accumulation, activation, and degranulation[55]	Present,[21,22] also on mast cell granule[22]; tryptase or trypsin does not activate rat peritoneal mast cells, but PAR-2AP does[21]; trypsin increased rat paw edema, possibly by mast cell histamine release[55]			
Eosinophils	Infiltration[18]	Activation and airway infiltration[17]; superoxide production and degranulation[26]	Present[26]		Superoxide production, degranulation, and increased response to IL-5[13]
Neutrophils	Not present[3]; thrombin has no effect[56]	Present[3]; expression of adhesion molecule CD11b[57]			
Alveolar macrophages	Present, function not defined[16]	Present, function not defined[16]			
Monocytes and dendritic cells	Present[3]; GM-CSF increases and IL-4 decreases expression[40]	Increases GM-CSF and decreases IL-4 expression[40]	GM-CSF increases and IL-4 decreases expression[40]		
Lymphocytes	T- and B-cell proliferation, increased IL-2 receptor, augments CD3 and IL-2 response[41]; Ca^{++} mobilization[58]	Present; function not defined[3]; Ca^{++} mobilization[58]; increased production of IgE to ovalbumin; mechanism not defined[17]			Increased total and specific IgE production[19,20]
Airway smooth muscle	Contraction[30]	Increased methacholine response in mice[17]; increased Ca^{++} in human subjects[7]; contraction in human subjects, with increased response to histamine; contraction enhanced by indomethacin[31,32]; contraction[30]; effect not mediated by PAR-2 in guinea pigs[59]; DNA synthesis and proliferation[31,50,51]			

Table continued on next page

TABLE I. *(continued)*

Cell	PAR-1	PAR-2	PAR-3	PAR-4	Cysteine protease
Endothelial cells	Factor Xa causes cytokine production of IL-6, IL-8, MCP-1, E-selectin, VCAM-1, and ICAM-1 in endothelial cells by unknown pathway, not PAR[27] (but one study[28] indicated it is PAR-1); thrombin produces IL-6 and IL-8[2]; TNF-α has a biphasic effect on PAR-1; IFN-γ and LPS had no effect[60]; but PAR production of IL-6 is enhanced by LPS and TNF-α[61]; P-selectin produced[29]	Present; production increased by IL-1 and TNF-α; stimulation elicits proliferation[3]; stimulation produces IL-6, IL-8, and NF-κB[62]			
Vascular smooth muscle	Mitogenesis[63]; constriction[2]				
Fibroblast precursors	Development and collagen production[44]	Development and collagen production[44]			
Fibroblasts	Mitogenesis, PDGF production[48,64]; vascular endothelial growth factor[49]; mitogenic effect mediated by activation of PDGF or connective tissue growth factor receptors[43]	Proliferation[45]; production of IL-8 and MCP-1[47]; chemotaxis and collagen production[46]			
Neurons	Reduced nociception[5]; production of substance P and VIP[65]	Present and might release substance P and VIP[5]			

COX-2, Cyclooxygenase 2; *VCAM-1*, vascular cell adhesion molecule 1; *ICAM-1*, intercellular adhesion molecule 1; *NF-κB*, nuclear factor for κ chains in B lymphocytes; *MCP-1*, monocyte chemotactic protein 1; *VIP*, vasointestinal peptide.

PAR activation of smooth muscle itself causes contraction. Stimulation of PARs generates metalloproteinase 9, an enzyme that disrupts junctions between cells.

Antigen-presenting cells express PAR-1 and PAR-2. The expression is increased by GM-CSF and decreased by IL-4.[16,40] Tryptase increases the production of TNF-α, IL-6, and IL-1 from human PBMCs.[67] Although exogenous proteases are clearly involved in the control of IgE production, the details of lymphocyte function are still preliminary.[17] Coagulation factor Xa stimulation of PAR-1 engenders proliferation of both T and B lymphocytes and augments CD3-dependent lymphocyte proliferation and expression of IL-2 receptor. At suboptimal concentrations, ligation of PAR-1 costimulates lymphocyte proliferation in the presence of IL-2.[41] Any effect of protease stimulation on IL-4 and IL-13 production remains to be defined. Using a mouse model, Kheradmand et al[42] found that fungal protease allergens delivered to the airways elicited a direct T_H2 IgE allergic response, but nonprotease antigens, such as ovalbumin, did not; they required priming with a remote adjuvant to overcome airway tolerance. Addition of a protease prevented this tolerance and induced an allergic response in the airway to the nonprotease allergen. These authors suggest that exogenous proteases might be required to overcome the innate resistance to development of T_H2 activation and allergic inflammation.

Proteases are critically involved in the angiogenesis and scarring of wound healing.[43] Thus they are likely to be an important part of the pathways for airway remodeling. The edema, angiogenesis, and fibrosis of the nasal mucosa that characteristically occurs in patients with chronic hyperplastic rhinitis and polyps represent an especially intriguing area for research. Endothelial cell response to PAR-1 opens up intercellular spaces and generates selectins, chemokines, and adhesion molecules,

TABLE II. Stimuli of PARs

PAR-1	PAR-2	PAR-3	PAR-4	Other protease stimuli
Thrombin[2,77]	Airway trypsin-like protease from bronchial epithelial cells[38]; bovine or porcine pancreatic trypsin[77]	Thrombin[2,77]	Thrombin[2,77]; trypsin[77]	Der f 1[13,14,20]
Chymotrypsin (chymase).[8]	Mast cell tryptase[77]			*Aspergillus* species[36,37,71-74]
Coagulation factor Xa[48,76]	Coagulation factor Xa^2; neutrophil proteases PR3, elastase, and cathepsin G[47] Der p 3 and Der p 9[39,70]			*Alternaria* species[36,75]

PR3, Proteinase 3.

resulting in inflammatory cell infiltration.[2,3,28] PDGF is one of the products of PAR stimulation in epithelial cells.[34] Stimulation of PAR-1 and PAR-2 promotes the maturation, proliferation, and collagen production of fibroblast precursors and mature lung and dermal fibroblasts.[44-47] Stimulation of PAR-1 of mature fibroblasts promotes production of the growth factors PDGF and vascular endothelial growth factor.[48,49] Mast cells are especially abundant in the airway smooth muscle of asthmatic patients and are presumably associated with disordered airway function.[68] Increase in mast cells is significant not for only the increased contractility of the smooth muscle but also because tryptase is a potent mitogen for airway smooth muscle, acting through pathways other than PAR-2.[50] There is, however, no information about the possible production of epidermal growth factor and the possible contribution of PARs to mucus gland hypertrophy. However, PAR-2 peptide agonists do stimulate mucus production by salivary and gastric glands.[69]

The research cited above has been limited to assays for the specific cells and substances mentioned and should be considered as intriguing and sometimes conflicting preliminary information. These data can by no means be considered complete and definitive about PARs. Of course, PARs are not the only receptors for these complex events. Furthermore, it remains to be investigated how various other signal transduction pathways are interrelated with those stimulated by proteases.

PROTEASES THAT ACTIVATE PARs

Thrombin produced during coagulation at sites of vascular injury is the primary endogenous intercellular serine protease agonist of PAR-1, PAR-3, and PAR-4. Trypsin and mast cell tryptase are the endogenous stimuli for PAR-2 (Table 2).[2,8,13,14,36-39,47,48,70-77] Airway epithelium, like gastrointestinal mucosa, is an important source of trypsin.[33,38]

Tryptase activation of PARs is much more complex than activation of neuromuscular receptors, such as β-adrenergic receptors. First, there are subtypes of tryptase that have different effects.[78] For example, α_1-tryptase recruits eosinophils and increases airway reactivity; β_1-tryptase recruits neutrophils but does not increase airway reactivity.[79,80] Second, tryptase activity can be inhibited by heparin released at the same time.[81,82] Third, the activity of tryptase depends on the sites of glycosylation of PAR-2 and the presence of sialic acid in the surrounding membrane.[82,83] Fourth, some of the effects of tryptase, particularly proliferation of smooth muscle, occur from the activation of protein kinases through pathways other than PAR-2.[50,51]

Tryptase isolated from human lung mast cells stimulates DNA synthesis, expression of intercellular adhesion molecule 1, and IL-8 release in a human epithelial cell line.[84] Tryptase also stimulates chemotaxis and collagen production of fibroblasts.[46,85] β-Tryptase produces contraction of isolated airway smooth muscle cells but not PGE_2. Contraction is inhibited by indomethacin.[31] Tryptase and other PAR-2 agonists induce proliferation of human airway smooth muscle cells, although this effect involves pathways other than PARs.[50,51] In general, the concentrations of tryptase required to elicit its effects *in vitro* are similar to those in the tissues after IgE-mediated release from mast cells. It seems likely that mast cell chymase (chymotrypsin) can also activate these receptors, at least PAR-1, although the evidence is still incomplete.[8] Chymase does enhance the skin wheal response to histamine.[86]

Kauffman[87] has reviewed the effect of proteases in airborne allergens on the respiratory mucosa. Although the receptors for these proteases have not yet been characterized, it is highly probable that the primary basis for this response is activation of PARs or similar molecules. In 1989, Ino et al characterized Der p 1 as a cysteine protease, and this activity has been confirmed in Group 1 allergens from the other species of mites that cause allergy.[88-91] Group 3 mite allergens are serine proteases.[92-96] Additionally, mite extracts contain elastase, chymotrypsin-like protease, and other proteases.[93,97] Mite serine proteases increase vascular permeability and detach epithelial cells.[98] Mite extracts stimulate airway epithelial cells to produce IL-8 and GM-CSF; the stimulation was blocked by cysteine and serine protease inhibitors.[39,70,99] After a short phase of cytokine production, Der p 1, like tryptase,

causes detachment of the epithelial cells, and cytokine production stops.[87,100] An elastase from mites degranulates mast cells.[99] The cysteine proteases from mites (Der p 1 and Der f 1) stimulate production of PGD_2 in airway epithelial cells and activate eosinophils.[13,101] The proteolytic activity of Der p 1 is inhibited by specific inhibitors of both serine and cysteine proteases.[102] Their stimulation of epithelial cells to produce IL-6 and IL-8 appears to be at least in part the result of activation of PAR-2.[14] Other investigators have reported that its production of IL-8 in epithelial cells results from activation of the extracellular signal regulated kinase 1/2, mitogen-activated protein kinase, and activating peptide 1 through activation of some other receptor, but not PAR-2.[103]

Fungi, especially *Aspergillus* and *Alternaria* species, produce large amounts of proteases that directly induce IL-8 production and subsequent epithelial cell detachment.[36,71,87,100] This effect is inhibited by cysteine protease inhibitors but not by serine protease inhibitors or α_1-antitrypsin. As mentioned above, proteases enhance IgE production to nonprotease allergens.[42] The production of fungal proteins with protease activity depends on the presence of proteins, especially collagen, in the culture medium.[104] Fungal extracts prepared on synthetic media do not contain all the allergens, especially proteases, produced by fungi growing in their natural environment or on the respiratory mucosa. Many pollen extracts contain proteases, and respiratory tract bacteria, *Pseudomonas* species, and *Staphylococcus* species also produce proteases. There are, however, no data on the possible effects of enzymes from these sources.

ROLE OF PROTEASES IN IgE-MEDIATED ALLERGIC DISEASES

After consideration of these widespread effects of stimulation of protease receptors by both endogenous and exogenous enzymes, it is clear that they are critically involved in the pathophysiology of allergic respiratory diseases. A simplified scenario can be considered as follows: FcεRI-activated release of tryptase from allergen-activated mast cells in the airway lumen contributes to the late phase of the allergen response by stimulating epithelial cells to release metalloproteinase 9 and open up tight junctions promoting allergen penetration into the submucosa. Possibly the stimulated epithelial cells release trypsin, another stimulus of PAR-2. Alternatively, the epithelial cells could be activated by airborne proteases from molds, mites, or pollens. Activation of other cells in the airway by endogenous and exogenous proteases increases production of IgE antibody and enhances infiltration of eosinophils, basophils, neutrophils, monocytes, and lymphocytes. Smooth muscle contraction is enhanced, nerves are made more reactive, and airway responsiveness is increased.[81] Eosinophils and mast cells are degranulated and stimulated to produce inflammatory molecules, such as nitric oxide, major basic protein, leukotrienes, histamine, and mast cell tryptase itself. Mast cell tryptase is likely to be especially important in the late phase of the allergic response.[105] The tryptase inhibitor APC 366 reduced the airway response to allergen challenge in a pig model.[106]

Although these effects of proteases leave little doubt that they have a significant role in the pathophysiology of allergic diseases, there are many issues that still require a great deal of investigation. Some of these effects are likely to be consistent and important, and others are likely to be inconsistent or minor. First, what are the details of the function of mast cell tryptase and chymase? Does human mast cell PAR-2 respond to tryptase? Do mucosal and connective tissue mast cells have differing activities? Do tryptase and chymase released from mast cell granules after allergen binds to FcεRI further amplify the reaction in other mast cells? What is the role of basophil granule proteins?

Second, are there significant genotypic differences in PARs, especially PAR-2, and if so, do these differences help explain differences in the occurrence, type, and severity of IgE-mediated disease in different individuals? Possible genotypic differences in PARs have not been investigated, although the effect of glycosylation on the activity of recombinant PAR-2 raises the possibility that genetic differences could produce differences in response.[107] Are there other factors that enhance the action of PARs through cross-talk between signal transduction pathways?

Third, is the enhanced production of IgE the result of production of IL-4 and IL-13? If, as likely, it is, what are the intercellular and intracellular pathways involved?

Fourth, what is the role of exogenous proteases? Do airborne proteases elicit significant respiratory disease through their enzymatic action? Do they enhance the IgE response to themselves or to other allergens? Does the fact that *Alternaria* species is a strong producer of proteases account for the frequency and severity of allergy to *Alternaria* species?[108] Do proteases from *Aspergillus* species growing on respiratory epithelium account for the exceptionally brisk immune response of allergic bronchopulmonary aspergillosis that includes not only IgE and IgG antibody but also cytotoxic granulomas? Might fungi germinating on airway epithelium in other allergic diseases contribute to their pathogenesis? Assays for these proteases from *Alternaria* and *Aspergillus* species in the air or in tissue fluids are quite feasible, and therefore these questions are answerable.

Fifth, what is the role of α_1-antitrypsin and other antitrypsins? As long as 25 years ago, Rudolph et al[109] found a correlation between the severity of nasal allergen challenge and the amount of endogenous protease inhibitor in nasal secretions. Heterozygous α_1-antitrypsin deficiency is more common in asthmatic patients than in control subjects, especially in patients with the aspirin triad.[110-116] Der p 1 inactivates α_1-antitrypsin.[117] However, α_1-antitrypsin does not inhibit tryptase or *Aspergillus fumigatus* proteases.[71,118] Secretory leukocyte protease inhibitor, a product of Clara cells, is another

naturally occurring antitrypsin that also blocks tryptase,[105,119,120] and it blocks the pulmonary response in an animal model of asthma.[66,118] There is no information about possible genetic deficiencies in this protein.

Sixth, what treatment is effective in reversing the effects of PARs? As yet, there is no direct information about pharmacologic control of PAR stimulation in allergic disease. Glucocorticoids inhibit expression of PAR-2 and production of GM-CSF by epithelial cells.[52] However, the increased expression of PAR-2 on epithelial cells of asthmatic subjects was not inhibited by inhaled glucocorticoids.[15] Because the cascade for PAR stimulation of gene transcription includes nuclear factor κB lymphocytes, glucocorticoids should also be expected to inhibit many of their effects. β-Adrenergic agonists and theophylline, which increase intracellular cyclic AMP levels, can be expected to inhibit the effects mediated by Ca^{++}. No specific PAR antagonists are yet available for treatment, although lapidated peptides and peptidemimetic antagonists are under development.[121-123]

ROLE OF PROTEASES IN ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

This complication of asthma and cystic fibrosis caused by *Aspergillus fumigatus* and related fungi growing in the bronchi is characterized by exuberant production of IgE and IgG antibodies. Levels of total serum IgE are extremely high, and not all of it is specific antibody to *Aspergillus* species antigens. The pathology includes pulmonary infiltrates of eosinophilic pneumonitis, granulomatous central bronchiectasis, and segmental pulmonary fibrosis.[124] *Aspergillus* species produces proteases.[36,71,72-74] These proteases can desquamate epithelial cells and stimulate IL-6 and IL-8 production.[36] *Aspergillus* species proteases also drive growth factor release from epithelial cells and are possibly responsible for the central bronchiectasis.[125] A question that stands out in glaring prominence is what is abnormal about the response in the few patients in whom this disease develops and why it is especially common in individuals with cystic fibrosis. Respiratory antileukoprotease is fungicidal and plays a role in innate immunity to fungi.[126] Possible deficiency of this protein has not been investigated.

ROLE OF PARs IN INTRINSIC ASTHMA, HYPERPLASTIC RHINITIS, AND POLYPS: THE ASPIRIN TRIAD

It has been almost 80 years since Francis Rackemann introduced the term *intrinsic asthma*, but the cause and pathogenesis of the syndrome are little better understood now than they were then. Consideration of the data about the effect of stimulation of cells of the airway by proteases offers a lead to understanding the mechanisms of the disease, if not its cause. There is a clear association between asthma, hyperplastic eosinophilic rhinitis, and polyps, and about a third of patients react severely to aspirin (ie, the aspirin triad).

Ponikau et al[127] have reported that 96% of nasal cultures of patients with chronic rhinosinusitis are positive for common fungi, particularly *Alternaria*, *Aspergillus*, and *Cladosporium* species.[128] Healthy control subjects had similar frequencies of positive cultures, but the patients with chronic hyperplastic rhinitis had hyphae in the mucus of 86% of surgical histologic specimens, indicating that the fungi were growing on the mucosa. Although the total serum IgE level was increased in a third of the patients, IgE or skin test results specific to fungi were positive in less than half of the patients. The authors viewed these positive IgE test results as recognition of the presence of fungi growing in the nose and not the cause of the disease. Subsequently, they reported that extracts of mucus and nasal tissue from these patients attracted peripheral blood eosinophils in a tissue culture and that the eosinophils from patients with chronic rhinosinusitis were more responsive than those from healthy control subjects.[129] Proteins in extracts of molds, especially *Alternaria* species, induced activation, IL-8 production, and degranulation of normal eosinophils through a pathway different from IL-5.[75] In confirmation of the importance of fungal colonization as the cause of the disease, the Mayo group has reported that nasal lavage with antifungal agents reverses the inflammatory thickening of the mucosa.[130,131]

Fungal proteases offer a plausible explanation for these observations.[36] The unique development of hyperplastic rhinitis and polyps with the typical eosinophilia, edema, angiogenesis, and fibrosis, as well as the increased local production of nonspecific IgE, could well be the effect of the activation of PARs by proteases produced locally by fungi growing on the mucus membrane. Of course, this explanation still does not account for the fact that these patients' responses to the fungi are strikingly different from those of the normal population. Is it possible that their protease receptors are different? Are they lacking some aspect of innate immunity to fungi? Furthermore, it is of interest that bronchial relaxation from PAR-2–induced production of PGD_2 in epithelial cells is inhibited by indomethacin, whereas the direct effect of PAR stimulation of smooth muscle cells is contraction that is enhanced by indomethacin.[31-33] Investigation of the role of fungal proteases in chronic hyperplastic rhinitis and asthma is in its infancy or, more exactly, still in utero.

POSSIBLE ROLE OF PARs IN THE RESPIRATORY DISEASES RELATED TO DAMP MOLDY BUILDINGS

Rhinitis, asthma, and other respiratory diseases have been linked to exposure to mites and molds growing in damp or water-damaged buildings.[132-134] Most of the data supporting this link with molds have been based on fungal cultures. Assay for fungal (or mite or bacterial) proteases

might be a much more effective means of correlating environmental exposure to disease.

SUMMARY AND CONCLUSIONS

Mast cell tryptase, long adrift unknown on the sea of our knowledge, has at last found its port of call, delivering an invaluable cargo of information. It stimulates PAR-2. PARs originally investigated for their role in coagulation and wound healing are also critically important in inflammation. They are widely distributed, particularly located on the cells involved in allergic diseases. Activation of PAR-2 stimulates respiratory epithelium to produce chemokines, cytokines, eicosanoids, and metalloproteinases that disrupt the tight junctions that bind epithelial cells to each other and to the basement membrane. It amplifies IgE production and the response of other mast cells to FcεRI stimulation. It recruits and activates inflammatory cells and degranulates eosinophils. It increases the contractile response of airway smooth muscle and, perhaps most importantly, remodels the airways with smooth muscle hypertrophy and fibroblast proliferation. Presumably tryptase, particularly β-tryptase, can stimulate PAR-2 to elicit most of these effects, although some of the actions of tryptase, notably smooth muscle hypertrophy, are mediated by a different mechanism.[51] Of course, these effects occur in parallel with the well-known similar effects of allergens and serve to amplify the reactions. Less information is available about PAR activation by mast cell chymase, but it has some of the same effects as tryptase. It activates PAR-1 and probably plays a special role in fibrosis.

Exogenous proteases from mites and molds are equally important stimuli that appear to act through different uncharacterized receptors. They amplify IgE production, degranulate eosinophils, and can cause allergic inflammation, even in the absence of IgE. In a mouse model simultaneous exposure to exogenous proteases is required to overcome airway tolerance for T_H2 activation and IgE production by nonprotease antigens. If this observation is extended to human subjects, it could help explain the timing and case selection of sensitization. Doubtless proteases produced by *Aspergillus* species to support its growth are responsible for the exuberant IgE, IgG, and granulomatous responses of allergic bronchopulmonary aspergillosis. Similar proteases from molds germinating on the respiratory mucosa have recently been implicated in the pathogenesis of chronic hyperplastic rhinitis and polyps and, by extension, intrinsic asthma. Finally, proteases from mites and fungi growing in damp, water-damaged buildings might be the basis for the increased prevalence in these buildings of rhinitis, asthma, and other respiratory diseases.

This review of the present state of knowledge about the protease stimulation of cell responses in allergic diseases has deliberately included many hypotheses and questions. Future research promises to be very productive in promoting our understanding of the pathogenesis of allergic respiratory diseases and pointing the way to new therapies.

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