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EDITORIAL

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Proteases and Protease-activated receptors signalling: at the crossroads of acquired and innate immunity

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Protease-activated receptors (PARs) are seven-transmembrane G-protein coupled receptors activated by serine proteases (Fig. 1). Proteases cleave the extracellular N-terminus of the molecule to expose a new tethered ligand, which in turn binds and activates the cleaved receptor. Four PARs have been cloned and each has a unique cleavage site amino acid sequence. Tryspin activates PAR2 and PAR4. Thrombin activates PAR1 and PAR3. Of particular interest for allergic disease, mast cell tryptase activates PAR2.

Much that is known about PARs and their function in the respiratory system comes from studies in the lower airways. All four PARs are expressed in airway epithelium and smooth muscle. PAR2 expression is increased in the asthmatic epithelium [1]. In isolated mouse airways, activation of epithelial PAR1, PAR2 and PAR4 by the proteases trypsin and thrombin, as well as by specific activating peptides corresponding to each receptor's tethered ligand amino acid sequence, cause airway relaxation via release of a cyclooxygenase product, probably prostaglandin E_2 (PGE_2) [2, 3]. PAR2 activation also causes relaxation of isolated rat, guinea pig and human bronchi, and induces bronchodilation in mice *in vivo* [2]. On the other hand, thrombin stimulates airway smooth muscle contraction, likely by activation of airway smooth muscle PAR1 [4]. Activation of PAR1, PAR2 and PAR4 stimulates IL-6, IL-8/CXCL8 and PGE_2 release from airway epithelial cells [5, 6]. Activation of PAR2 also induces airway epithelial cell release of granulocyte-macrophage-colony stimulating factor (GM-CSF), eotaxin/CCL11 and matrix metalloproteinase (MMP)-9 [7–9]. Basolateral stimulation of PAR2 receptors in mouse and human airways results in phospholipase C and Ca^{2+}-dependent inhibition of amiloride-sensitive Na^+ conductance and stimulation of both luminal Cl^- channels and basolateral K^+ channels, leading to a secretory response [10]. PAR2 activation interrupts E-cadherin adhesion and compromises the airway epithelial barrier [11].

PARs are also present on mast cells, eosinophils, neutrophils, alveolar macrophages, monocytes and lymphocytes [12]. Thrombin and the PAR1 activating peptide induce β-hexosaminidase, IL-6 and MMP-9 release from mouse bone marrow mast cells, as well as mast cell adhesion to fibronectin [13]. Trypsin induces activation and superoxide release from human eosinophils through PAR2 [14]. PAR2 stimulation of peripheral blood monocytes induces Ca^{2+} flux and production of IL-1β, IL-6, and IL-8/CXCL8 [15]. Stimulation of human peripheral monocytes and monocyte-derived macrophages with thrombin or PAR1 activating peptide triggers expression of monocyte chemoattractant protein (MCP)-1/CCL2 [16]. Thrombin, trypsin and the PAR2 activating peptide induce calcium flux in human T cell lines [17].

The abundant effects of PAR2 activation on airway cell and leucocyte function are consistent with the notion that PAR2 plays a critical role in the pathogenesis of allergic airways disease. To test this, Schmidlin et al. [18] examined the response to ovalbumin (OVA) sensitization and challenge in PAR2 knockout mice, as well as mice undergoing intranasal administration of the PAR2 activating peptide SLIGRL-NH_2. Compared with wild-type animals, eosinophil infiltration was inhibited by 73% in mice lacking PAR2 and increased by 88% in mice overexpressing PAR2. Similarly, compared with wild-type animals, airway cholinergic responsiveness was diminished 38% in mice lacking PAR2 and increased by 52% in mice overexpressing PAR2. PAR2 deletion also reduced IgE levels to OVA sensitization by fourfold compared with those of wild-type animals. Thus, PAR2 significantly contributes to the development of acquired immunity and allergic inflammation in the airways.

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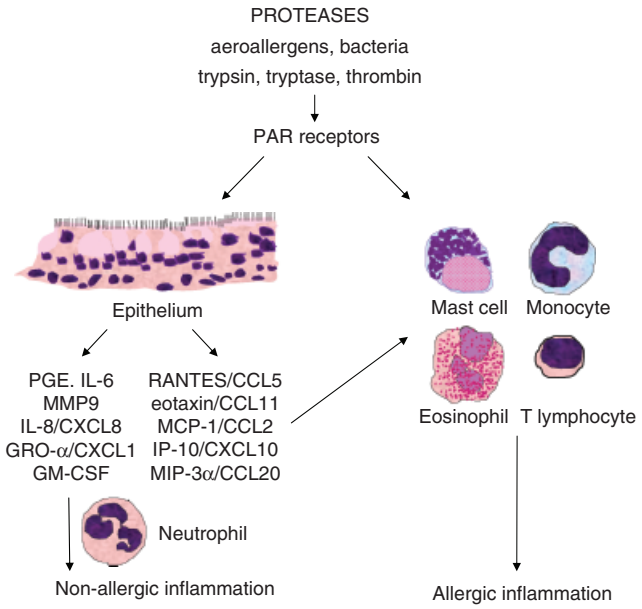

Fig. 1. Proteases, including those from aeroallergens and bacteria, induce respiratory epithelial cell cytokine expression by protease-activated receptors (PAR)-dependent and independent mechanisms. Protease-induced expression of C–X–C chemokines and granulocyte-macrophage-colony stimulating factor (GM-CSF) is sufficient to initiate neutrophilic inflammation. In addition, proteases directly stimulate leucocyte activation, thereby enhancing allergic responses. Finally, protease stimulation induces the epithelium to produce C–C chemokines capable of attracting eosinophils, monocytes and T cells to the airway. Thus, proteases and PAR receptors may hold a central role in both the innate and acquired immune responses. MCP, monocyte chemoattractant protein; PGE, prostaglandin; MMP, matrix metalloproteinase; GRO, growth related oncogene; RANTES, regulated on activation normal T cell expressed and secreated; MIP, macrophage inflammatory protein; IP, inducible protein 10.

It is well-known that aeroallergens carry intrinsic protease activity capable of disrupting tight junctions and increasing transepithelial allergen delivery [19–21]. More recently, it has been established that airborne allergens activate airway epithelial cell PARs. Two major dust mite antigens with serine protease activity, Der p 3 and Der p 9, activate PAR2 and induce PAR2-mediated release of GM-CSF and eotaxin [8]. The cysteine protease Der p 1 induces airway epithelial cell Ca^{2+} flux and IL-6 expression via activation of PAR2 [22]. Cockroach serine proteases increase IL-8/CXCL8 expression in human bronchial epithelial cells via activation of a PAR2/extracellular-signal-regulated kinase/nuclear factor for IL-6 pathway [6, 23, 24]. Thus, aeroallergens with protease activity may induce a non-allergic, innate inflammatory response via the activation of PAR2 and release of pro-inflammatory cytokines.

In mouse models of asthma, type I allergens such as OVA require priming with adjuvants remote from the lung to overcome airway tolerogenic mechanisms that ordinarily preclude allergic responses to inhaled allergens. However, type II allergens such as *Aspergillus fumigatus*, *Aspergillus oryzae* and ragweed pollen require neither remote priming nor additional adjuvants to overcome airway tolerance and elicit robust allergic lung disease. Kheradmand et al. [25] showed that proteolytic activity is both necessary and sufficient for overcoming airway tolerance and induction of pulmonary allergic disease, suggesting that PAR-dependent and independent stimulation of the airway epithelium by serine proteases may be critical for the allergic airway response. However, as aerosolized OVA is sufficient for IgG2a responses and IgE tolerance [26], proteases probably do not permit allergic responses simply by enhancing antigen presentation via the degradation of tight junction proteins. Instead, protease activation of epithelial cell PARs may facilitate allergic responses to OVA by inducing the expression of chemokines required for maximal leucocytic activation and infiltration. Like proteases, airway GM-CSF transgene expression allows aerosolized OVA to induce allergic sensitization in mice [27]. In addition to airway eosinophilia, mice expressing GM-CSF show greater numbers of antigen-presenting dendritic cells and macrophages, as well as an expansion of both CD4 and CD8 cells. As noted above, activation of PAR2 stimulates epithelial cell production of GM-CSF [7], an activator of granulocytes and macrophages. Finally, it has recently been shown that Der p 1 induces chemotaxis of monocyte-derived dendritic cells via the bronchial epithelial cell production of IL-8/CXCL8, CXCL10, MCP-1/CCL2, CCL5 and CCL20 [28].

Comparatively little is known about the expression of PARs in the nasal epithelium or their potential role in seasonal allergic rhinitis (SAR), chronic rhinosinusitis, bilateral nasal polyposis or other conditions. Nasal mucosal biopsies of patients with SAR show increased epithelial cell expression of PAR2, as well as increased numbers of mast cells and eosinophils in the nasal mucosa [29]. Stimulation of nasal epithelial cells with fungi induces PAR2 and PAR3 mRNA expression [30]. Immunoreactive PAR2 colocalizes with tachykinins in trigeminal neurons innervating the nasal mucosa, suggesting that trypsin and mast cell tryptase stimulation of PAR2 in tachykinergic neurons could trigger neurogenic inflammation [31].

In this issue of *Clinical and Experimental Allergy*, Rudack et al. [32] show that PAR2 expression is increased in nasal biopsies from patients with chronic rhinosinusitis without nasal polyps, a disease caused by impaired sinus drainage and characterized by tissue infiltration with lymphocytes and neutrophils [33]. Based on this neutrophil predominance, chronic rhinosinusitis resembles non-eosinophilic asthma, a disease characterized by neutrophilic airway inflammation [34–36]. PAR2 expression was increased to a lesser extent in patients with bilateral nasal polyposis, an entity characterized primarily by eosinophilic inflammation. Consistent with this, stimulation of cultured nasal epithelial cells with either

PAR2 activating peptide, trypsin or Staphylococcal serine proteases induced G_i protein- and NF-κB-dependent expression of the neutrophil chemoattractants IL-8/CXCL8 and growth-related oncogene-αCXCL1, but not the eosinophil and T cell chemoattractants eotaxin/CCL11, CCL5 or CCL17. Together, these data are consistent with the notion that, in patients with chronic rhinosinusitis, bacterial proteases induce neutrophilic inflammation via an innate immune response including activation of PAR2 and subsequent expression of CXC chemokines. In other words, the specific signalling outcomes of PAR2 activation determine the character of nasal inflammation in chronic rhinosinusitis. Accordingly, therapeutic interventions directed against the PAR2/G_i/NF-κB pathway may be beneficial in the treatment of this sinus disease.

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