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Role of protease-activated receptors in airway function: a target for therapeutic intervention?

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Abstract

Protease-activated receptors (PARs) are G-protein-coupled, seven transmembrane domain receptors that act as cellular enzyme sensors. These receptors are activated by the proteolytic cleavage at the amino terminus, enabling interaction between the newly formed “tethered ligand” and the second extracellular loop of the receptor to confer cellular signalling. PARs can also be activated by small peptides that mimic the tethered ligand. In the respiratory tract, PARs may be regulated by endogenous proteases, such as airway trypsin and mast cell tryptase, as well as exogenous proteases, including inhaled aeroallergens such as those from house dust mite faecal pellets. Immunoreactive PARs have been identified in multiple cell types of the respiratory tract, and PAR activation has been reported to stimulate cellular mitogenesis and to promote tissue inflammation. Activation of PARs concurrently stimulates the release of bronchorelaxant and anti-inflammatory mediators, which may serve to induce cytoprotection and to minimise tissue trauma associated with severe chronic airways inflammation. Furthermore, airway inflammatory responses are associated with increased epithelial PAR expression and elevated concentrations of PAR-activating, and PAR-inactivating, proteases in the extracellular space. On this basis, PARs are likely to play a regulatory role in airway homeostasis, and may participate in respiratory inflammatory disorders, such as asthma and chronic obstructive pulmonary disease. Further studies focussing on the effects of newly developed PAR agonists and antagonists in appropriate models of airway inflammation will permit better insight into the role of PARs in respiratory pathophysiology and their potential as therapeutic targets. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Airway inflammation; Asthma; Cytoprotection; PAR; Protease; Thrombin receptor

Abbreviations: ASM, airway smooth muscle; BAL, bronchoalveolar lavage; CGRP, calcitonin gene-related peptide; COPD, chronic obstructive pulmonary disease; COX, cyclo-oxygenase; ECM, extracellular matrix; hPAR, human protease-activated receptor; MMP, matrix metalloprotease; MT-SP1, membrane-type serine protease 1; mPAR, murine protease-activated receptor; NK, neurokinin; NO, nitric oxide; NOS, nitric oxide synthase; PAR, protease-activated receptor; PG, prostaglandin.

Contents

1. Introduction	240
2. <i>Protease-activated receptor</i> gene structure	241
3. Molecular biology of protease-activated receptors	241
4. Pharmacology of protease-activated receptor agonist and antagonists.	242
4.1. Protease-activated receptor agonist peptides	242
4.2. Potency of protease-activated receptor agonist peptides	242
4.3. Selectivity of protease-activated receptor agonist peptides.	243
4.4. Protease-activated receptor antagonists	243
5. Protease-activated receptor distribution in the airways	243

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6.	Potential activators of protease-activated receptors in airway tissue	244
6.1.	Mast cell tryptase	245
6.2.	Airway trypsin	245
6.3.	Trypsin-like proteases	245
6.4.	Membrane-bound airway proteases	245
6.5.	Matrix metalloproteases	246
6.6.	Coagulation-related proteases	246
6.7.	Proteases from haemopoietic cells	246
6.8.	Respiratory allergens and other exogenous proteases	246
7.	Potential inactivators of protease-activated receptors in airway tissue	247
8.	Role of protease-activated receptors on airway function	247
8.1.	Effect of protease-activated receptor activation on airway smooth muscle tone in vitro	247
8.2.	Effect of protease-activated receptor activation on pulmonary resistance in vivo	248
9.	Role of protease-activated receptors in airway remodelling and inflammation	248
9.1.	Effects of protease-activated receptor activation on resident airway cells during homeostasis	248
9.2.	Effects of protease-activated receptor activation on airway inflammatory cells	249
9.3.	Effects of protease-activated receptor activation during airway inflammation	250
10.	Protease-activated receptors in airway disease	250
10.1.	Effect of airway inflammation on protease-activated receptor expression	250
10.2.	Interrelationship between proteases, protease inhibitors, and protease-activated receptors in the airway	250
10.3.	Protease-activated receptors: pro-inflammatory or cytoprotection in the airway?	251
11.	Conclusion	251
	Acknowledgements	251
	References	252

1. Introduction

Protease-activated receptors (PARs) are a novel group of G-protein-coupled, seven transmembrane domain receptors that enable cells to monitor extracellular proteolytic activity. Unlike conventional agonist-receptor systems, PAR activa-

tion is dependent on the specific cleavage of the amino-terminus of the receptor. The newly formed amino-terminus then functions as a “tethered ligand,” docking and interacting with the second extracellular loop of the PAR to trigger the sequence of events involving G-protein dissociation that confers receptor activation (for recent overviews, see Mac-

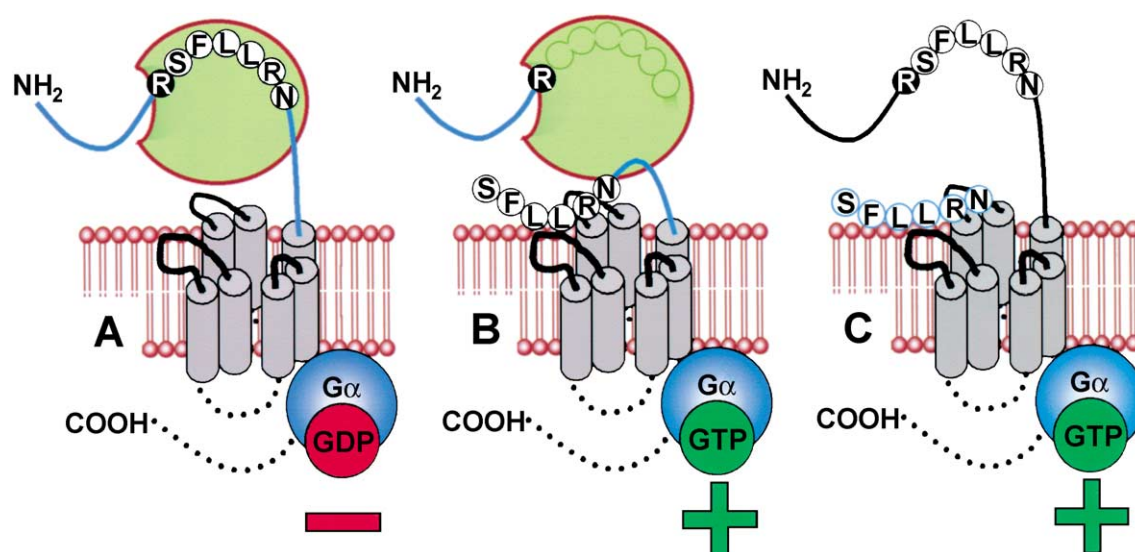


Fig. 1. Proposed mechanism for PAR activation utilising thrombin and hPAR1 as a model. The activity of hPAR1 is negligible in its native form (–). **A:** Docking of thrombin on the PAR extracellular amino (NH₂) terminal exodomain and subsequent cleavage at the activation site (R/S). **B:** The newly exposed “tethered ligand,” the SFLLRN... motif, interacts with the second extracellular loop of the receptor to confer intracellular signalling through the activation of G-proteins (+). **C:** Activation of PAR using a synthetic peptide (SFLLRN) identical to the tethered ligand sequence.

farlane et al., 2001; O'Brien et al., 2001) (Fig. 1). Thus, PARs provide one avenue through which various cellular and tissue responses to catalytically active proteases may be explained.

Thus far, 4 PARs, denoted chronologically by their discovery as PAR1, PAR2, PAR3, and PAR4, have been cloned and characterised. While thrombin (EC 3.4.21.5, activating PAR1, PAR3, and PAR4) and trypsin (EC 3.4.21.4, activating PAR2 and PAR4) have been proposed as the preeminent endogenous activators of PARs, there is accumulating evidence to suggest that other proteases (such as neutrophil-derived cathepsin G) and inhaled enzymes (such as those present in house dust mite faecal pellets) may also cleave and activate PARs (Sambrano et al., 2000; Sun et al., 2001). These proteases may play a more biologically relevant role at tissue sites where thrombin and trypsin are less likely to be present. It is now well established that peptide analogues that mimic the amino acid sequence of the PAR-tethered ligand can also activate PAR1 [such as SFLLRN for human PAR₁ (hPAR₁)], PAR₂ (SLIGKV), and PAR₄ (GYPGQV), but, interestingly, not PAR₃ (TFRGAP) (Macfarlane et al., 2001) (see Table 1). These agonist peptides display a greater selectivity for their respective receptors than do trypsin and thrombin, although the utilisation of both agonist peptides and the proteases as research tools has proven very valuable in establishing the biological activity of the PARs in vivo.

Table 1
General properties of PARs

PAR subtype	PAR gene localisation	Cleavage site: tethered ligand sequence ¹	Potential endogenous activator ²	Potential endogenous inactivator ³
PAR1	(h) 5q13 (m) 13D2	(h)...R ⁴¹ ↑SFLLRN... (m)...R ⁴¹ ↑SFLLRN...	Thrombin Trypsin Granzyme A	Cathepsin G Elastase Plasmin Proteinase 3
PAR2	(h) 5q13 (m) 13D2	(h)...R ³⁴ ↑SLIGKV... (m)...R ³⁸ ↑SLIGRL...	Trypsin Tryptase Factor Xa proconvertin MT-SP1	Cathepsin G Plasmin Proteinase 3
PAR3	(h) 5q13 (m) 13D2	(h)...K ³⁸ ↑TFRGAP... (m)...K ³⁷ ↑SFNGGP...	Thrombin	Cathepsin G Elastase (h)
PAR4	(h) 19p12 (m) 8B3.3	(h)...R ⁴⁷ ↑GYPGQV... (m)...R ⁵⁹ ↑GYPGKF...	Thrombin Trypsin Cathepsin G (h)	–

An overview of the general properties of PARs. (h) human PAR; (m) mouse PAR.

¹ Alphabets denote the one-letter symbol abbreviation for amino acids, and are numbered accordingly, beginning from the amino terminus of the translated protein sequence. Appropriate cleavage site (↑) by activating proteases.

² Exposes a newly formed amino terminal exodomain (for example, SFLLRN... in hPAR₁) which behaves as a “tethered ligand” and self-activate PARs. Synthetic peptides analogous to the tethered ligand (such as SFLLRN for hPAR₁) can also activate its corresponding receptor for PAR₁, PAR₂, PAR₄, but not PAR₃.

³ Other endogenous proteases can cleave carboxyl to the activation site and inactivate PAR.

Although proteases have been implicated in the pathogenesis of multiple lung diseases, such as asthma and chronic obstructive pulmonary diseases (COPD) (Rennard et al., 1991; Barnes, 1998; Mautino et al., 1999), the homeostatic role of airway proteases and their interactions with PARs remain less well established. PARs localised in the respiratory system appear to regulate airway smooth muscle (ASM) tone, airway inflammation, and tissue repair and regeneration. In this review, we will outline the pharmacological profile of the four cloned PARs, the physiological function of PARs in the airway, the interaction between lung proteases and PARs, and the potential implications of PARs in respiratory diseases.

2. Protease-activated receptor gene structure

The 4 PAR genes share a similar structure, as they are comprised of 2 exons, with exon 1 and exon 2 encoding a signal peptide and the mature receptor protein, respectively (Kahn et al., 1998a). Interestingly, the PAR₁, PAR₂, and PAR₃ genes are clustered within the same locus in both the mouse (13D2) and the human (5q13) chromosome, whereas the PAR₄ gene is located on a separate chromosome (8B3.3 in the mouse; 19p12 in the human) (Schmidt et al., 1997; Kahn et al., 1998a; Xu et al., 1998) (see Table 1). The collocation and similarity in the PAR gene structure suggested that these genes arose from multiple duplications of an ancestral gene (Kahn et al., 1998a). More recently, PAR₁ and PAR₂ gene polymorphisms have been described (Compton et al., 2000; Park et al., 2000). For example, male carriers of a 13-bp insertion polymorphism in the 5' untranslated region of PAR₁ demonstrate reduced risks of venous thromboembolism, suggesting a potential protective effect of this allele against this condition (Arnaud et al., 2000a), but not against other cardiovascular diseases such as myocardial infarction (Arnaud et al., 2000b). Similarly, a single nucleotide polymorphism in the translational sequence of PAR₂ resulted in a functionally distinct PAR₂ with reduced sensitivity to proteolytic and peptidic activators (Compton et al., 2000). The full potential for the functional consequences of PAR polymorphisms in homeostasis and pathophysiology has yet to be examined, and remains an important field for future investigation.

3. Molecular biology of protease-activated receptors

The activation of PARs by thrombin is initially dependent on the docking of the protease to a high-affinity, acidic hirudin-like sequence located on the amino terminal exodomain of the receptor. Although such a docking process has yet to be examined for other proteolytic PAR activators such as trypsin, the binding of thrombin to the PAR (specifically PAR₁ and PAR₃) appears to be important to confer highly potent receptor cleavage and activation by

thrombin [with the concentration required to elicit a half-maximal response (EC_{50}) approximating 50 pM in *Xenopus* oocytes overexpressing PAR1] (Vu et al., 1991b). In addition, mutations at either the docking sites of thrombin (Myles et al., 2001) or the receptor (Vu et al., 1991b) can significantly attenuate thrombin potency in PAR-mediated cell signalling, suggesting that the docking of thrombin to the PAR is an important process to enable effective cleavage to expose the tethered ligand.

One other critical molecular event in PAR-mediated cellular signalling involves the interaction between the newly exposed tethered ligand with the second extracellular loop of the receptor. Single amino acid substitutions in the second extracellular loop of both PAR1 and PAR2 have been shown to diminish PAR signalling by thrombin and trypsin, respectively (Al-Ani et al., 1999a; O'Brien et al., 2000), suggesting the integrity of the second extracellular loop is critical for intracellular signalling through its interaction with the exposed tethered ligand. Interestingly, recent studies using site-directed mutagenesis of the second extracellular loop have generated a receptor that is responsive to thrombin, but not to PAR agonist peptides (Blackhart et al., 2000), despite unaltered binding kinetics of the agonist peptide to the receptor. These findings indicate that the intrinsic efficacy and the docking mechanism of both the tethered ligand and the PAR agonist peptides are mechanistically different (Hollenberg, 2000), and that the protease and the agonist peptide may generate dissimilar functional responses. Consistent with this, PAR agonist peptide-sensitive, but thrombin/trypsin-insensitive, responses were observed in rat pulmonary arteries (Roy et al., 1998). Conversely, studies in guinea pig bronchi have demonstrated trypsin-sensitive, but not PAR2 agonist peptide-sensitive, responses (Carr et al., 2000). Such discrepant results have been variously attributed to PAR agonist peptide degradation by tissue-derived proteases (Coller et al., 1992), inactivation of PAR proteolytic activators by the release of endogenous serine protease inhibitors (serpins) (Turgeon & Houenou, 1997), as well as the possibility of multiple receptor activation by nonselective proteolytic PAR activators, such as thrombin and trypsin (Table 1). However, these findings may also be indicative of the existence of PAR subtypes or a hitherto unknown PAR (Carr et al., 2000; Hamilton et al., 1998; Roy et al., 1998; Ruef et al., 2000).

4. Pharmacology of protease-activated receptor agonist and antagonists

4.1. Protease-activated receptor agonist peptides

Synthetic peptide fragments representing the amino terminus of the tethered ligand of PAR1, PAR2, and PAR4, but not PAR3, are able to activate their respective receptor (Table 1). The optimal length of such receptor-

activating peptides (previously called thrombin receptor-activating peptides) range from a minimum of 5–14 amino acid residues, with some studies showing greater potency exhibited by shorter, rather than longer, activating peptides (Hollenberg et al., 1992; Vassallo et al., 1992; Kawabata et al., 1999b). Conversely, the reversal of the two amino acids residing in the amino terminus of the PAR1 and PAR2 activation peptide (i.e., SFLLRN to FSLLRN, and SLIGRL to LSLIGRL) consistently yielded a functionally inactive peptide in transfected cells and tissue bioassays, and frequently have been used experimentally in conjunction with an active peptide as a negative control (Vassallo et al., 1992; Hollenberg et al., 1997; Al-Ani et al., 1999b; Lan et al., 2000, 2001). Interestingly, the partially scrambled murine PAR4 (mPAR4) agonist peptide YGPGKF is fully functional (Lan et al., 2000). Although the reason for this finding is unknown, GYPGKF and YGPGKF have been shown to exhibit heterologous cross-desensitisation in the isolated mouse trachea (Lan et al., 2000), suggesting that these two agonist peptides interact with a common (PAR4) signal transduction pathway. Alternate negative peptides, such as GYPGFK and GAPGKF, have since been reported (Kahn et al., 1998b; Faruqi et al., 2000; Lan et al., 2000).

4.2. Potency of protease-activated receptor agonist peptides

Various modifications of the PAR agonist peptides have been reported to alter their potency. Acetylation of the amino terminus generally inactivates the agonist peptide, an effect possibly due to an alteration in the ionisation state of the serine amino group, which may prevent agonist peptide binding to the receptor (Coller et al., 1992; Hollenberg et al., 1993, 1997). Conversely, amidation of the carboxyl terminus of the agonist peptide tends to increase agonist potency by ~10-fold, a feature that has been attributed to the removal of the negative charges of the carboxyl terminal motifs (Hollenberg et al., 1993, 1996b; Al-Ani et al., 1999b). The increase in the structural similarity between the agonist peptide and the tethered ligand by altering the carboxyl and amino terminus of the agonist peptide may explain, in part, the observed increases in agonist peptide potency (Hollenberg, 2000). In addition, the potency of the PAR1 agonist peptide can also be increased by substituting the serine residue for the β amino acid isoserine (i.e., SFLLRN to (iso-S)FLLRN) through reducing the susceptibility of the peptide to peptidase degradation (Coller et al., 1993). Although insignificant PAR agonist peptide hydrolysis occurs *in vitro* by most accounts (Hollenberg et al., 1993; Laniyonu & Hollenberg, 1995; Al-Ani et al., 1999b), some studies reported that the co-administration of peptidase inhibitors, such as amastatin and phosphoramidon, with PAR agonist peptides is necessary to prevent PAR agonist peptide hydrolysis and to increase the sensitivity of PAR-mediated responses (Coller et al., 1992;

Hollenberg et al., 1996a; Ahn et al., 2000; Saifeddine et al., 2001).

4.3. Selectivity of protease-activated receptor agonist peptides

Although individual PARs are responsive to their corresponding agonist peptide based on its tethered ligand motif, comparison of the tethered ligand amino acid sequences reveals significant homology between different species of a PAR (such as mPAR1 and hPAR1) and between individual PARs (especially PAR1 and PAR2) (see Table 1). For example, in addition to activating the hPAR1, the agonist peptide SFLLRN has been reported to cross-activate both hPAR2 and mPAR2 (Blackhart et al., 1996; Maryanoff et al., 2001). Furthermore, the mPAR2 agonist peptide SLIGRL can activate both mPAR2 and hPAR2 (Blackhart et al., 1996; Maryanoff et al., 2001), although this latter agonist peptide does not activate PAR1 or PAR4 (Blackhart et al., 1996; Lan et al., 2000; Maryanoff et al., 2001). Although a pharmacological cross-desensitisation assay has demonstrated a lack of heterologous cross-reactivity by mPAR1, mPAR2, and mPAR4 agonist peptides (SFFLRN, SLIGRL, and GYPGKF, respectively) (Lan et al., 2000), these findings demonstrate the potential for some synthetic agonist peptides to activate multiple PARs in tissue samples obtained from various species, and validated the need to modify such agonist peptides that confer receptor, and perhaps species, selectivity. The *Xenopus*-human hybrid peptide TFLLR is an example of a modified, selective PAR1 agonist that does not activate PAR2 (Hollenberg et al., 1997). Similarly, an alanine substitution in mPAR4 (from GYPGFK to AYPGKF) yielded a highly potent PAR4 agonist peptide that does not activate other hPARs (Faruqi et al., 2000). The further development of such selective PAR agonist peptides is critical when assessing individual PAR activity in isolated tissues and whole animal studies.

4.4. Protease-activated receptor antagonists

The development of potent, selective, and stable PAR agonists has been paralleled by the development of PAR antagonists. Several peptidomimetics, such as 3-mercaptopropionyl-Phe-Cha-Cha-Arg-Lys-Pro-Asn-Asp-Lys-amide (Mpa-peptide) and BMS-200661, potentially antagonise PAR1, although their effects on other PARs are not known (Seiler et al., 1995; Bernatowicz et al., 1996). More recently, RWJ-58259 and the non-peptidic compound SCH-79797 have been reported to block PAR1, but not other PARs (Andrade-Gordon et al., 1999; Ahn et al., 2000). RWJ-58259 elicited a modest antithrombotic effect in a guinea pig model of thrombosis, suggesting that PAR1 antagonists may be of potential use in the treatment of cardiovascular obstructive diseases without invoking bleeding complications (Pakala et al., 2000; Andrade-Gordon et al., 2001).

5. Protease-activated receptor distribution in the airways

PARs are expressed in multiple tissue types in various organs (Bohm et al., 1996b; Ishihara et al., 1997; D'Andrea et al., 1998; Xu et al., 1998; Kahn et al., 1999; Jenkins et al., 2000; Bretschneider et al., 2001). Within the respiratory tract, PARs are expressed by airway epithelial and smooth muscle cells, as well as endothelial and vascular smooth muscle cells (D'Andrea et al., 1998; Ricciardolo et al., 2000; Knight et al., 2001; Schmidlin et al., 2001b) (see Table 2). PARs are also present in the terminal bronchial epithelium and Type II pneumocytes of the peripheral lung (Saifeddine et al., 2001), and migratory cells of the airway wall, such as lung macrophages, mast cells, granulocytes, and lymphocytes, also express immunoreactive PARs (Nelken et al., 1992; Mari et al., 1996; Howells et al., 1997; D'Andrea et al., 2000; Miike et al., 2001). However, the specific distribution and expression of each PAR subtype in the airway remains to be delineated. Some cells of the respiratory tract, such as the ASM (PAR1, PAR2, PAR3) and mast cells (PAR1, PAR2), co-express multiple PARs (Hauck et al., 1999; D'Andrea et al., 2000) (see Table 2). Since different PARs exhibit not only different protease specificity, but also varying intracellular signalling pathways (Shapiro et al., 2000; Wang et al., 2002), expression of multiple PARs on a single cell type may permit selective responses to various proteases in the extracellular space of the airway. Furthermore, immunoreactive PAR1 and PAR2 staining was higher on the bronchial epithelium than the

Table 2
General localisation of PARs in the airway

	PAR1	PAR2	PAR3	PAR4
PAR localisation in airway tissue				
Trachea	Epithelium, ASM, mast cells	Epithelium, ASM, vascular smooth muscle, endothelium		
Lower bronchus	Epithelium, ASM	Epithelium, ASM, vascular smooth muscle, endothelium, fibroblasts, bronchial glands, leukocytes	Epithelium	Epithelium
Peripheral lung	Mast cells	Type II pneumocytes, mesothelial cells, mast cells	+	+
PAR expression by airway (cultured) cells				
Epithelial cells	+	+	+	+
ASM cells	+	+	+	
Airway fibroblasts		+		

Summary of PARs in airway tissue and cultured cells. +, the presence of PAR mRNA (reverse transcription-polymerase chain reaction, Northern blots, in situ hybridisation) and protein expression (immunocytochemistry, flow cytometry).

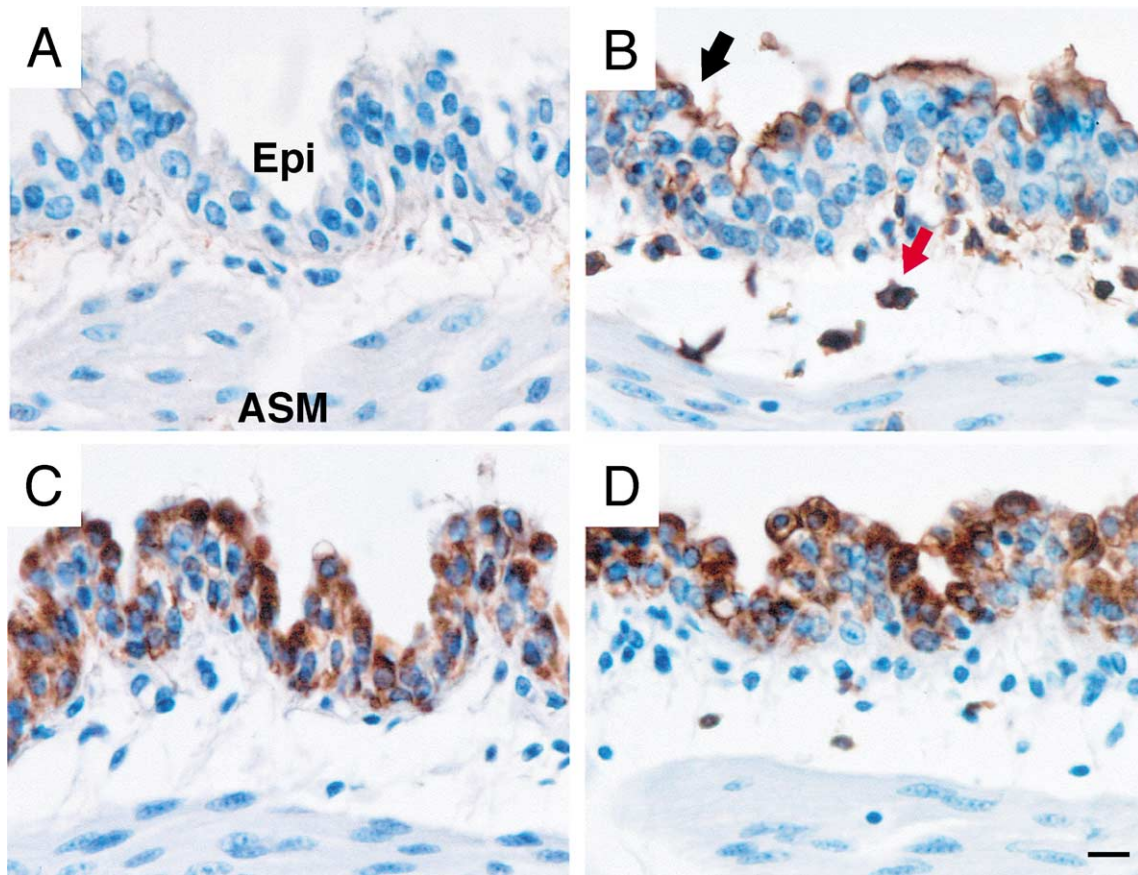


Fig. 2. Photomicrographs of tracheal sections isolated from CBA/CaH mice. **A:** Slight PAR2 immunoreactivity in the tracheal epithelium (Epi) and ASM from sham-infected mice. **B:** Increased immunoreactive PAR2 in the apical part of the tracheal Epi (black arrow) in influenza virus-infected mice, characterised by the presence of PAR2-expressing mononuclear inflammatory cells (red arrow). **C** and **D:** COX-2 immunoreactivity in the tracheal Epi from sham-infected mice (**C**) and virus-infected mice (**D**). Scale bar=10 μ m for all figures.

ASM (Jenkins et al., 2000; Knight et al., 2001). As the potency of PAR activators is dependent, in part, on the level of receptor expression (Cicala et al., 1999b; Kawabata et al., 2001c), variable levels of PAR expression by individual cell types may reflect the capability to monitor for various concentrations of PAR activators in the airway.

Immunohistochemical studies have also indicated that PARs are expressed on both the cell membrane and in the cytosol of airway cells (Bohm et al., 1996b; D'Andrea et al., 1998, 2000; Cocks et al., 1999a; Hauck et al., 1999; Chow et al., 2000; Jenkins et al., 2000; Ricciardolo et al., 2000; Knight et al., 2001; Saifeddine et al., 2001) (see Fig. 2). Such an intracellular reserve pool of PARs may be indicative of rapid PAR translocation from the cytosol to the cell surface and may prevent prolonged PAR desensitisation after receptor activation (Bohm et al., 1996a; Shapiro et al., 1996; Molino et al., 1997b). Similarly, the finding that the short-circuit current can only be altered by basolateral, but not apical, PAR2 agonist peptide in bronchial epithelial cells also suggests the possibility that PAR-coupling and intracellular signalling mechanisms may be dependent on the specific location of the receptor on the cell membrane

(Danahay et al., 2001). Thus, intracellular trafficking and translocation of PARs to the cell membrane in the airway is likely to be a continuous and highly regulated process, and individual PARs may possess different coupling and signal transduction pathways in pulmonary cells.

6. Potential activators of protease-activated receptors in airway tissue

Although cleavage of the appropriate arginine or lysine residue of the PAR amino terminal exodomain represents the critical event in exposing the tethered ligand, there are other factors to consider when assessing the physiological relevance of various proteases as activators of PARs in the airway. Although cleavage specificity and catalytic efficiency of the protease is highly dependent on the "specificity pocket" of the catalytic site, the non-catalytic domains of the protease may play an important role by influencing substrate accessibility and alignment into the specificity pocket (Perona & Craik, 1997). Other considerations include the presence of potential binding sites on PARs that

may enhance protease-to-receptor binding affinity (such as the hirudin-like sequence on hPAR1 and mPAR3 to permit thrombin binding and potent signalling) (Vu et al., 1991b; Nakanishi-Matsui et al., 2000), as well as the ability of the protease to guide the tethered ligand to interact with the extracellular activating domains of the receptor (Brass et al., 1994). Moreover, the multiple factors involving the overall biology of the protease, including its biochemical pathways for synthesis, release, and degradation, as well as the bioavailability of the protease in the extracellular space and its susceptibility to endogenous protease inhibitors, have to be thoroughly assessed in order to provide supporting evidence for the candidate protease to play a physiologically relevant role through PAR activation in vivo.

6.1. Mast cell tryptase

Tryptase (EC 3.4.21.59) is a large homotetrameric heparin-bound protease secreted from degranulating mast cells. Tryptase has been shown to cleave substrate peptides analogous to the PAR2 amino terminal exodomain (Fox et al., 1997; Smith et al., 2000), and can activate phosphoinositide hydrolysis and mitogenesis in PAR2-transfected cells (Mirza et al., 1997; Molino et al., 1997a). Tryptase cannot activate cells expressing uncleavable PAR2 mutants where the appropriate amino acids at the activation site have been altered (Mirza et al., 1997) or cells that previously had been desensitised to PAR2 agonist peptides (Schechter et al., 1998). While the overall size of tryptase (approximating 150 kDa) (see Zhang & Timmerman, 1997; Sommerhoff et al., 2000) may impede its mobility in the extracellular space, detectable levels of this protease have been recovered from induced sputum and bronchoalveolar lavage (BAL) fluid (Louis et al., 2000; Krawiec et al., 2001). BAL samples from these latter studies were obtained from healthy subjects, suggesting that tryptase can be synthesised and released by mast cells into the extracellular spaces, including the airway lumen, despite the absence of significant airway inflammation. Collectively, these findings strongly suggest that tryptase can regulate homeostatic function in the airway by activating PAR2.

However, in the absence of appropriate stabilising factors such as heparin, tryptase spontaneously dissociates into inactive monomers (Hallgren et al., 2000; Huang et al., 2000; Sommerhoff et al., 2000). The ability of tryptase to cleave PAR2 is also dependent on the level of receptor glycosylation (Compton et al., 2001). Moreover, tryptase may inactivate PAR2 at high concentrations (100 nM) (Molino et al., 1997a). Thus, while tryptase can cleave and activate PAR2, various other factors may diminish its role as a physiologically relevant PAR activator.

6.2. Airway trypsin

Monomeric, 24-kDa trypsin (EC 3.4.21.4) previously was considered to be exclusively localised within the gastro-

intestinal tract. However, it recently has been found in other tissues, including human bronchial epithelial cells, as well as vascular endothelial cells (Kawano et al., 1997; Koshikawa et al., 1997; Cocks et al., 1999a; Danahay et al., 2001). Since trypsin is a well-established activator of PAR2 and PAR4 in vitro and can cleave other PARs, albeit less effectively (Parry et al., 1996; Altrogge & Monard, 2000), airway trypsin has the potential to activate PARs in the respiratory tract in vivo. However, several important issues regarding airway trypsin remain to be investigated, including the mechanism(s) of activation of the pro-enzyme (zymogen), as well as the regulatory pathway governing its synthesis and degradation.

6.3. Trypsin-like proteases

Secretory trypsin-like proteases expressed by airway cells that may activate PARs include tryptase Clara, a monomeric 30-kDa trypsin-like protease secreted by bronchial epithelial Clara cells (Kido et al., 1992), and miniplasmin, a catalytically active by-product of plasmin (EC 3.4.21.7) localised in bronchial epithelial cells (Murakami et al., 2001). Both tryptase Clara and miniplasmin have been shown to cleave and activate viral envelope glycoproteins, and play an important role in potentiating the pathogenicity of respiratory tract viruses, such as Sendai virus and influenza (Kido et al., 1996; Murakami et al., 2001). Another novel trypsin-like protease in the airways is human airway trypsin, which is expressed in submucosal glands of the human bronchus and trachea, and can cleave and activate neutrophil-derived matrix metalloproteases (MMPs) (Yasuoka et al., 1997; Tsukada et al., 2001). However, despite the presence of such catalytically active and biologically important proteases in the vicinity of airway PARs, the ability for such trypsin-like enzymes to cleave the amino terminal exodomain of PARs has not been examined yet.

6.4. Membrane-bound airway proteases

Ecto-enzymes, which are transmembrane proteins with an extracellular proteolytic domain, have been reported to be constitutively expressed and synthesised by various airway cells. Examples of such membrane-bound ecto-proteases in the airway include endopeptidases (for example, neutral endopeptidase, EC 3.4.24.11), aminopeptidases (such as aminopeptidase M, EC 3.4.11.2), and carboxypeptidase (EC 3.4.17) (for a review, see van der Velden & Hulsmann, 1999). The degradative activities of these ectoenzymes in the airway are relatively well characterised, and substrates for these proteases include neuropeptides, vasoactive peptides, and interleukins (ILs) such as IL-6 (van der Velden & Hulsmann, 1999). Although there is some evidence to suggest that aminopeptidase M can cleave PAR1 agonist peptides in vitro (Coller et al., 1992), its ability to cleave and activate

PARs in vivo is not clear. Recently, membrane-type serine protease 1 (MT-SP1; also called epithin) has been identified and is abundantly expressed in the lungs (Kim et al., 1999; Takeuchi et al., 1999). Soluble MT-SP1 has been demonstrated to cleave and activate PAR2 (Takeuchi et al., 2000). Since membrane-anchored thrombin and Factor Xa can cleave and activate PARs (Bono et al., 2000; Nakanishi-Matsui et al., 2000; Riewald & Ruf, 2001), it is possible for other membrane-bound proteases such as MT-SP1 to regulate airway homeostasis through autocrine PAR activation. Alternatively, it has also been suggested that such ectoenzymes can be solubilised through exocytosis or cleavage of its membrane-bound domain to provide an alternate pathway for such proteases to cleave PARs, and circumvent the potential predicament of steric hindrance and protease-to-substrate proximity (Ehlers & Riordan, 1991; van der Velden & Hulsmann, 1999).

6.5. Matrix metalloproteases

Zinc-dependent MMPs (EC 3.4.24) represent a family of extracellular matrix (ECM)-degrading proteases that include collagenases, gelatinases, stromelysins, and membrane-type MMPs (Shapiro & Senior, 1999). MMPs are actively secreted by the airway epithelium, alveolar macrophages, and neutrophils during airway inflammation and remodelling (Hoshino et al., 1998; Rajah et al., 1999; Shapiro & Senior, 1999), and play an important role in regulating airway ECM remodelling and inflammation through the degradation of ECM molecules (Vu & Werb, 2000). MMPs can also modulate the inflammatory process by cleaving biologically active peptides, such as endothelins and calcitonin gene-related peptide (CGRP) (Fernandez-Patron et al., 1999, 2000b). However, the ability of MMPs to activate PARs, to date, have not been reported.

6.6. Coagulation-related proteases

Thrombin is a well-established activator of PAR1, PAR3, and PAR4 (Vu et al., 1991a; Altrogge & Monard, 2000), but other proteases involved in coagulation may also cleave and activate PARs. For example, Factor Xa (EC 3.4.21.6) activates PAR1 and PAR2 (Kawabata et al., 2001b; McLean et al., 2001; Riewald & Ruf, 2001), in part by utilising its effector cell protease-1 receptor as a cofactor (Bono et al., 2000; Camerer et al., 2000). Proconvertin (tissue factor/Factor VIIa complex) can also cleave and activate PAR2 (Camerer et al., 2000). However, other proteases that mediate coagulation, including plasmin, tissue plasminogen activator (EC 3.4.21.68), urokinase (EC 3.4.21.73), plasma kallikrein (EC 3.4.21.34), as well as activated Protein C (EC 3.4.21.69), exhibit minimal activation of PARs (Parry et al., 1996; Fox et al., 1997; Altrogge & Monard, 2000) in vitro.

Although some resident cells of the airway, including alveolar macrophages and the pulmonary endothelium, can synthesise tissue factor (Chapman et al., 1988; Schultze & Roth, 1993; Nakstad et al., 1998), currently there is little evidence to suggest that coagulation-related proteases are abundantly secreted by airway resident cells during homeostasis. However, the findings that PAR activation contributes to vasodilatation and oedema (Kawabata et al., 1998, 1999a) suggests that stimulation of pulmonary vascular PARs can promote an influx of proteases, which, in turn, can activate PARs localised in the airway. Animal models of respiratory oedema have similarly demonstrated the presence of protein exudates into the airway lumen (Olivenstein et al., 1997), and coagulation-related proteases such as thrombin have also been recovered from human sputum (Gabazza et al., 1999). Such proteases, presumably secreted as inactive zymogens, may be activated by the endothelium and alveolar macrophages, or circulating monocytes and neutrophils, in the airway (Nakstad et al., 1998; Hartwig et al., 1999). Collectively, these studies suggest coagulation-related proteases have little effect on the uninflamed airway, but can be recruited into the respiratory tract and activate airway PARs during pulmonary oedema and inflammation.

6.7. Proteases from haemopoietic cells

During inflammation, migratory inflammatory cells are recruited into the airways and contribute to tissue repair and remodelling through the secretion of various proteases into the extracellular spaces. Some of these secretory proteases are also known to activate PARs. For example, the T-lymphocyte-derived protease granzyme A (EC 3.4.21.78) can cleave the amino terminal domain of PAR1 (Suidan et al., 1994; Parry et al., 1996), and neutrophil-derived cathepsin G (EC 3.4.21.20) can activate cells expressing PAR1 and PAR4 (Sambrano et al., 2000). Although low, but detectable, levels of these proteases have been recovered in the BAL from healthy subjects (Sepper et al., 1995; Tremblay et al., 2000), these inflammatory cell-derived proteases probably play a minimal role in PAR-mediated homeostatic function, and impart a significant contribution to PAR activation only during airway inflammation.

6.8. Respiratory allergens and other exogenous proteases

Airborne allergens, such as those from house dust mite faecal pellets, fungi, pollen, and occupational dust, have been shown to stimulate the release of pro-inflammatory cytokines in vitro (Hassim et al., 1998; King et al., 1998; Wan et al., 1999), and can induce hyper-reactivity of the airways in vivo (Gavett & Koren, 2001; Riccioni et al., 2001). There is now evidence to suggest that these inhaled proteases may stimulate such responses through the activation of PARs. House dust mite serine

protease allergens from *Dermatophagoides pteronyssinus*, *Der p 3* and *Der p 9*, have been shown to interact with PAR2 (Sun et al., 2001). However, the interaction of other such exogenous proteases with PARs currently is unknown.

7. Potential inactivators of protease-activated receptors in airway tissue

By cleaving the carboxyl terminus to the activation site and removing the tethered ligand from the receptor, certain proteases have the potential to inactivate PARs and render the receptor refractory to activation by other proteases, such as thrombin and trypsin (Table 1). Plasmin, cathepsin G, and neutrophil proteinase 3 (EC 3.4.21.76) can inactivate PAR1 and PAR2 (Molino et al., 1995; Parry et al., 1996; Kuliopulos et al., 1999; Loew et al., 2000), while neutrophil elastase (EC 3.4.21.37) can inactivate PAR1 and PAR3 (Parry et al., 1996; Altrogge & Monard, 2000; Loew et al., 2000). Cathepsin G is also an inactivator of PAR3 (Cumashi et al., 2001). These results suggest that inactivation of the PAR is predominantly effected by coagulation-related proteases and proteases released from haemopoietic cells. In addition, the findings that protease inhibitors such as phosphoramidon and amastatin are able to potentiate the effects of PAR agonist peptides suggest that proteases such as MMPs and aminopeptidases can hydrolyse PAR agonist peptides (Coller et al., 1992; Hollenberg et al., 1996a; Ahn et al., 2000; Saifeddine et al., 2001) and, by the same token, may potentially cleave the tethered ligand of the PAR and inactivate the receptor. PAR inactivation provides a novel mechanism of receptor desensitisation independent of protease inhibition, receptor phosphorylation, or receptor internalisation (Shapiro et al., 1996; Coughlin, 1999; Tirupathi et al., 2000). Such a pathway may be biologically important in diseases involving airway inflammation, such as bronchiectasis and emphysema, when the levels of pro-inflammatory and coagulation-related proteases, such as neutrophil elastase and serum plasmin, are significantly elevated in the extracellular spaces (Sepper et al., 1995; Yoshioka et al., 1995).

Interestingly, some airway proteases can function as both activators and inactivators of PAR. For example, cathepsin G behaves as both an activator and inactivator of PAR1 in vitro (Loew et al., 2000; Sambrano et al., 2000; Cumashi et al., 2001). However, inactivation of PAR1 is thermodynamically favoured over cathepsin G-mediated PAR1 activation, with the latter only detectable at high receptor expression (Molino et al., 1995; Sambrano et al., 2000). Similarly, the PAR2 activators trypsin and tryptase can also inactivate its receptor at high protease concentrations (100 nM) (Molino et al., 1997a). Taken together, these data suggest a novel mechanism for such dual PAR activating-inactivating proteases to prevent an excessively vigorous or prolonged cellular stimulus.

8. Role of protease-activated receptors on airway function

8.1. Effect of protease-activated receptor activation on airway smooth muscle tone in vitro

Activation of PARs induces vascular smooth muscle relaxation from various isolated preparations, including coronary and mesenteric arteries, saphenous veins, as well as pulmonary vessels (Teschfamiar et al., 1993; Glusa et al., 1997; Fernandez-Patron et al., 2000a). PAR-mediated vascular relaxations are generally abolished either by the removal of the endothelium or by pretreating the preparation with a nitric oxide synthase (NOS) inhibitor, suggesting that activation of PARs induce relaxation through the release of endothelium-derived nitric oxide (NO) (Teschfamiar et al., 1993; Glusa et al., 1997; Fernandez-Patron et al., 2000a). There is accumulating evidence to show that PARs similarly modulate ASM tone in vitro. For example, activators of PAR2 and PAR4 primarily induces relaxation in isolated tracheal and bronchial rings obtained from various sources, including murine, rat, and guinea pig airways (Cocks et al., 1999a; Chow et al., 2000; Lan et al., 2000; Ricciardolo et al., 2000). These responses were generally dependent on the release of cyclo-oxygenase (COX)-derived relaxant prostanoids from the airway epithelium (Cocks et al., 1999a; Chow et al., 2000; Lan et al., 2000, 2001), with NO playing a lesser role (Ricciardolo et al., 2000). Conversely, activation of PAR1 evokes mainly contraction (Hauck et al., 1999; Carr et al., 2000; Chow et al., 2000; Saifeddine et al., 2001). As the contractile response to PAR1 activation is only marginally modulated by conventional pharmacological antagonists and tissue treatments such as epithelium removal, the mechanism for PAR1-mediated contraction in vitro has been attributed to direct activation of PAR1 localised on the ASM rather than the release of diffusible contractile factor(s) (Hauck et al., 1999; Carr et al., 2000; Chow et al., 2000; Saifeddine et al., 2001).

Interestingly, activation of some PARs is associated with biphasic responses in ASM tone. For example, in mouse isolated tracheal preparations, PAR1- and PAR4-mediated relaxations were preceded by transient contractions (Lan et al., 2000), whereas in guinea pig bronchus, PAR2-mediated contractions followed an initial transient relaxant response (Ricciardolo et al., 2000). Such biphasic responses are also observed in gastrointestinal tissue, such as the mouse gastric fundus (Cocks et al., 1999b) and the rat duodenal smooth muscle (Kawabata et al., 2000). These responses may represent the overall summated response induced by a single PAR agonist peptide on different cell types, with each cell type coupled to alternate transduction pathways (Cocks et al., 1999b).

PAR-mediated changes in ASM tone appear to differ significantly between species and the type of airway tissue examined. For example, PAR2 activation induces relaxation in isolated bronchial rings taken from the murine, rat, and

guinea pig airways (Cocks et al., 1999a; Chow et al., 2000; Ricciardolo et al., 2000), and conversely, stimulates contraction in human isolated bronchial rings (Schmidlin et al., 2001b; Chambers et al., 2001). Furthermore, PAR2-mediated relaxation in the rat bronchus, but not the rat trachea, is sensitive to indomethacin (Chow et al., 2000), suggesting that despite similar outcomes, PAR signal transduction pathways are likely to vary, depending on the anatomical location of the receptor. In summary, PARs modulate airway tone in vitro, and consistent with their cellular distribution, the functional response is dependent on the species examined, the cell and tissue type activated, and the specific cellular site of activation (Tables 2 and 3).

8.2. Effect of protease-activated receptor activation on pulmonary resistance in vivo

Studies recording lung function in vivo are likely to be more complex than isometric measurement of ASM tone in vitro, since indices of pulmonary resistance in vivo are dependent not only on the bronchial smooth muscle tone per se, but are also dependent on multiple contributive factors to airway obstruction, such as fluid and mucous secretion into the airway lumen, severity of the (experimental) trauma on tissue integrity, as well as thickness and compliance of the airway submucosa and parenchyma (Drazen, 1976; Postma & Kerstjens, 1998; Drazen et al., 1999; Leong & Huston, 2001). However, such studies examining the role of PARs in airway function are important and complimentary to the in vitro findings. Intravenous administration of PAR2 agonist peptides in the rat and guinea pig provided bronchoprotec-

tion against serotonin- and histamine-induced increases in pulmonary resistance (Cocks et al., 1999a; Cicala et al., 2001). The mechanism for bronchoprotection is not known, although it may be due in part to PAR2-stimulated inhibition of histamine-induced pulmonary leakage (Cicala et al., 2001). Conversely, Ricciardolo et al. (2000) reported that PAR2 agonist peptides induced a direct increase in guinea pig airways resistance, a response mediated by the release of contractile prostanoids and neuronal-derived tachykinins. PAR1 agonist peptide-induced bronchoconstriction in vivo, on the other hand, was dependent on the release of a platelet-derived contractile factor (Cicala et al., 1999a) (see Table 3).

9. Role of protease-activated receptors in airway remodelling and inflammation

9.1. Effects of protease-activated receptor activation on resident airway cells during homeostasis

Activation of PARs on airway cells can promote growth and can stimulate tissue repair and regeneration. Activation of PAR1 and PAR2 on lung fibroblasts and ASM cells induces mitogenesis (Panettieri et al., 1995; Trejo et al., 1996; Akers et al., 2000; Berger et al., 2001; Chambers et al., 2001), a response likely to be accentuated by PAR-stimulated release of mitogenic mediators such as platelet-derived growth factor from bronchial epithelial cells (Shimizu et al., 2000). PAR1 activation has also been reported to promote fibroblast proliferation and migration through the

Table 3

General effects of PAR activation in various cells and tissue types obtained from the respiratory tract

Assay types conducted	Cell/tissue systems examined	Biological responses to PAR activators in airway cells	Potential implication
Secretion of mediators	Bronchial epithelium, mast cells, lung fibroblasts, isolated mouse and guinea pig tracheal rings	↑ PDGF, CTGF ↑ Substance P, IL-6, IL-8, eotaxin, granulocyte-macrophage colony-stimulating factor ↑ MMP-9 ↑ Prostanoids (including PGE ₂), NO	Proliferation of fibroblasts and ASM cells Promotion of oedema and recruitment of inflammatory cells Airway remodelling Cytoprotective, anti-inflammatory, bronchorelaxants
Proliferation studies	Bronchial epithelium, lung fibroblasts, airway smooth muscle cells	Mitogenesis, procollagen synthesis	Stimulate proliferation of fibroblasts and airway smooth muscle cells, and promotes remodelling in submucosa
Isometric tension recording	Isolated tracheal rings, bronchial rings and lung parenchymal strips obtained from mice, rats guinea pigs, humans	Direct airway smooth muscle contraction Indomethacin-sensitive airway smooth muscle relaxation	Contributory role to airway hyperresponsiveness
In vivo lung function recording	Mechanically ventilated rats and guinea pigs	Increased lung resistance Inhibits effect of spasmogens	Bronchoprotection through release of inhibitory prostanoids Increased bronchial obstruction through release of Substance P and platelet-derived contractile factors Inhibition of leakage?

Summary of the effect of PAR activation in airway tissues. ↑, an increase in the quantity of mediators released. CTGF, connective tissue growth factor; PDGF, platelet-derived growth factor.

up-regulated expression of connective tissue growth factor, a mitogen (Chambers et al., 2000). Taken together, these studies suggest that activation of PARs in various cells is likely to stimulate mitogenesis in the airway.

PAR activation may also play a role in remodelling through the stimulated release of MMP-9 and procollagen from bronchial epithelial cells and lung fibroblasts, respectively (Chambers et al., 1998; Vliagoftis et al., 2000). Activation of PAR1 and PAR2 on epithelial cells, fibroblasts, mast cells, and eosinophils has also been reported to stimulate the secretion of chemokines, including IL-8, granulocyte-macrophage colony-stimulating factor, and eotaxin, as well as the pro-inflammatory mediators IL-6, Substance P, and eosinophil-derived neurotoxin (Gordon et al., 2000; Ludwicka-Bradley et al., 2000; Miike et al., 2001; Sun et al., 2001; Vliagoftis et al., 2001). Thus, activation of PARs on airway cells may trigger growth and tissue remodelling, and can indirectly promote recruitment, proliferation, and activation of various inflammatory cells.

It is currently unclear whether PAR activation of resident airway cells can elicit a significant inflammatory response. However, there is evidence from several *in vivo* animal models that support a pro-inflammatory role for PARs. Subplantar injections of PAR1 or PAR2 agonist peptides in the rat increased vascular permeability and induced oedema (Kawabata et al., 1998, 1999a; Vergnolle et al., 1999a, 1999b; Steinhoff et al., 2000), and recruited PAR2-expressing granulocytes to the site of peptide administration (Vergnolle, 1999; Vergnolle et al., 1999a; Steinhoff et al., 2000). PAR-stimulated increases in vascular permeability and oedema may be due, in part, to mast cell degranulation (Cirino et al., 1996; Kawabata et al., 1998, 1999a). In addition, PAR-induced oedema is slightly augmented by COX and NOS inhibitors (Kawabata et al., 1999a; Vergnolle et al., 1999b), suggesting that the concurrent PAR-stimulated release of prostanoids and NO may serve as a braking mechanism to prevent perpetual oedema and inflammation.

PAR-induced oedema has also been reported to be sensitive to repeated capsaicin administration, and can be inhibited with neurokinin (NK)-1 and CGRP-1 receptor antagonists (Steinhoff et al., 2000; de Garavilla et al., 2001). These findings suggest a mediator role for afferent nerve-derived tachykinins, such as Substance P and NKA, as well as the vasodilator CGRP, in PAR-induced oedema. Interestingly, PAR-stimulated granulocyte infiltration in these models was not inhibited by NK1/CGRP1 receptor antagonists, nor by COX/NOS enzyme inhibitors, and probably occurred through the release of other diffusible chemokines (Ludwicka-Bradley et al., 2000; Vliagoftis et al., 2001), as well as an up-regulation of adhesion molecules (Sugama & Malik, 1992; Shankar et al., 1994; Zimmerman et al., 1994; Kaplanski et al., 1998). Modulation of inflammatory cell trafficking by PARs has been further examined through the use of intravital microscopy, and PAR agonist peptides have been shown to reduce leucocyte velocity and to increase leukocyte adherence to capillaries (Zimmerman

et al., 1994; Vergnolle, 1999; Lindner et al., 2000) through the stimulated release of endothelial platelet-derived factor and an up-regulation of adhesion molecules, such as P-selectin and intercellular adhesion molecule-1 on endothelial cells (Sugama et al., 1992; Kaplanski et al., 1998; Vergnolle, 1999; Lindner et al., 2000). Thus, these studies demonstrate that oedema and inflammatory cell recruitment to the site of PAR activation can occur through the release of various epithelium- and endothelium-derived chemokines and up-regulation of adhesion molecules, while concurrently releasing negative feedback, regulators including prostanoids and NO.

9.2. Effects of protease-activated receptor activation on airway inflammatory cells

Mast cells are resident inflammatory cells, and mast cell-derived mediators have been implicated in immediate hypersensitivity responses (Bingham & Austen, 2000; Hart, 2001). PAR1 and PAR2 are expressed in mast cells of the tracheal submucosa and the peripheral lung (Chow et al., 2000; D'Andrea et al., 2000). However, the effects of PAR activation on mast cell function are not clear. The findings that PAR1-induced oedema in the rat hindpaw was abolished by the depletion of mast cell mediators with Compound 48/80 (Kawabata et al., 1998, 1999a) and that subdermal administration of PAR1 agonist peptides in rats depicted histological evidence of mast cell degranulation (Cirino et al., 1996; Umarova et al., 2000) suggest that PAR activation promotes oedema through mast cell activation and degranulation. In contrast, other studies showed little histological evidence of mast cell degranulation in rat tissues treated with PAR1 or PAR2 agonist peptides (Vergnolle et al., 1999a; Chow et al., 2000). The differences in these findings may be due to the different time points used in these studies to assess mast cell-mediated oedema (Vergnolle et al., 1999a). Additionally, PAR activation, unlike immunoglobulin E-mediated mast cell degranulation, may stimulate controlled secretion of selective mediators from mast cells (Gordon et al., 2000). Furthermore, the existence of a heterogeneous family of mast cells categorised by different granule content and tissue distribution may also contribute to these disparate findings (McNeil & Gotis-Graham, 2000).

Expression of PAR1 and PAR2 have also been detected in various inflammatory cells, including T-cells, eosinophils, and monocytes (Howells et al., 1993; Mari et al., 1994, 1996; Naldini et al., 1998; Fiorucci et al., 2001; Miike et al., 2001). Activation of PAR1 in monocytes has been reported to stimulate Ca^{2+} mobilisation, actin polymerisation, and cellular proliferation (Hoffman & Church, 1993; Joseph & MacDermot, 1993; Naldini et al., 1998) and to promote the release of the growth factor and chemokine monocyte chemoattractant protein-1 (Colotta et al., 1994). These studies suggest that PAR1 activators can stimulate recruitment and cellular proliferation in monocytes. Similarly, PAR2 agonist

peptides have been reported to alter the cellular structure in neutrophils (Howells et al., 1997; Lourbakos et al., 1998), and may regulate cellular motility and migration. In addition, activation of PAR2 on eosinophils induces superoxide anion production and cellular degranulation (Miike et al., 2001). Conversely, PAR2 agonist peptides inhibited the synthesis of interferon- γ and suppressed the expression of the adhesion molecule CD44 from activated T-cells (Fiorucci et al., 2001), suggesting that PAR activation may alternately stimulate or suppress inflammatory cell activity, depending on the inflammatory cell type and its state of activation.

9.3. Effects of protease-activated receptor activation during airway inflammation

Consistent with the findings that PAR agonist peptides can elicit a pro-inflammatory phenotype, PAR2-deficient mice have been shown to exhibit suppressed tissue oedema and a reduced inflammatory cell infiltrate in an animal model of contact dermatitis (Kawagoe et al., 2002). In addition, a greater number of inflammatory cells were recovered from the BAL in transgenic mice overexpressing PAR2 when sensitised with ovalbumin (Schmidlin et al., 2001b). However, under certain circumstances, PARs may mediate cytoprotection and minimise cellular damage induced by physical trauma and noxious stimuli that produces cellular stress. PAR1 activation prevented neuronal cell degeneration during oxygen and glucose deprivation (Vaughan et al., 1995; Striggow et al., 2000). Complement-induced endothelial cytolysis has been reported to be reduced, in part, by preactivating PAR1 (Lidington et al., 2000). In addition, pretreating rodents with PAR2 agonist peptides can minimise acid-induced damage in the gastrointestinal tract. This latter protective mechanism is dependent upon the release of endogenous CGRP and tachykinins from afferent neurones and reduced expression of T helper Type 1-type mediators, such as IL-2, interferon- γ , and tumour necrosis factor- α , and adhesion molecules such as CD44 (Fiorucci et al., 2001; Kawabata et al., 2001a). Similarly, infusion of PAR2 agonist peptides reduced ischaemic zones and produced significant recovery of myocardial function in the isolated rat heart after ischaemia (Napoli et al., 2000).

The effects of PAR activation during airway inflammation is currently unclear. Although intranasal administration of PAR2 agonist peptides has been shown to directly stimulate macrophage infiltration in the BAL of mice (Schmidlin et al., 2001a), other studies have demonstrated that PAR2 agonist peptides can conversely reduce lipopolysaccharide-stimulated neutrophilia in murine airways (Cocks & Moffatt, 2001). These findings collectively suggest that it is possible for PAR activation to stimulate anti-inflammatory mechanisms against various cytotoxic stimuli, and this digression from the predominantly pro-inflammatory phenotype observed by PAR activators may depend on the type of exogenous stimuli applied, the severity of the pre-existing

inflammatory trauma, the time point(s) of PAR agonist peptide administration, and the types of PARs activated.

10. Protease-activated receptors in airway disease

10.1. Effect of airway inflammation on protease-activated receptor expression

The airway epithelium is the first tissue of contact with inhaled particles, such as airborne aeroallergens and respiratory tract viruses, and as such, has been the subject of intense research interest. Biopsy specimens obtained from human subjects with α_1 -antitrypsin deficiency exhibited altered PAR2 distribution in the airway epithelium, and the bronchial epithelium of asthmatic subjects express elevated levels of PARs (Cocks & Moffatt, 2001; Knight et al., 2001). Tracheal sections obtained from mice infected with influenza virus showed increased epithelial PAR2 staining intensity at the site of infection (Lan et al., 2000) (see Fig. 2). Thus, these findings collectively suggest that elevated PAR expression may accompany pulmonary inflammation. Although changes in PAR expression previously have been reported to alter PAR agonist potency in other tissues (Cicala et al., 1999b; Kawabata et al., 2001c), the physiological significance of PAR up-regulation during airway inflammation is not understood yet, since virus-induced up-regulation of epithelial PAR2 did not alter the sensitivity of PAR2-mediated relaxation of the mouse trachea in vitro (Lan et al., 2000). Nevertheless, this increase in cellular PAR expression may reflect an alteration in cellular sensitivity to increased proteolytic PAR activators and inactivators in the extracellular space during respiratory diseases, such as asthma, bronchiectasis, and COPD (Sepper et al., 1997; Gabazza et al., 1999; Barnes, 2000; Louis et al., 2000).

10.2. Interrelationship between proteases, protease inhibitors, and protease-activated receptors in the airway

There is a significant body of evidence demonstrating the importance of exogenous protease inhibitors, endogenous serpins, and tissue inhibitors of metalloproteases regulating airway remodelling and inflammation. Inhibitors of MMP and elastase have been shown to attenuate neutrophil-directed acute lung injury (Carney et al., 1999; Rees et al., 1999), and aerosolised secretory leukocyte protease inhibitor prevented airway hyperresponsiveness in allergen-sensitised sheep, guinea pig, and mice (Wright et al., 1999). However, while such inflammatory cell-derived proteases may interact with PARs, the cellular effects resulting from PAR interaction can often be masked by catalytically important, but PAR-independent, responses. For example, the pathological feature of extravascular fibrin deposition in the lung by thrombin in adult respiratory distress syndrome and the destruction of alveolar parenchyma by elastase in COPD and bronchiectasis are unlikely to be due to direct

PAR activation (Barnes, 2000; Idell, 2001), suggesting that the observed bronchoprotective effect of exogenous protease inhibitors may not be due to the interactions of these agents with PARs. Hence, delineating which protease-induced actions are PAR-mediated and determining the pro-inflammatory or cytoprotective phenotype evoked by PAR activation in airway cells during the diseased state are both issues that need to be addressed when assessing the role of PARs in airway inflammation, and merit important consideration when reassessing the protease-antiprotease hypothesis of airway diseases (Gadek & Pacht, 1990; Shapiro, 1995; Knight et al., 1997).

10.3. Protease-activated receptors: pro-inflammatory or cytoprotection in the airway?

The inflammatory response is an important process for the clearance of exogenous toxins and virus/parasite-infected cells (Larsen & Holt, 2000). However, chronic inflammation in diseases such as asthma can contribute to airway wall remodelling and eventual irreversible airway hyperresponsiveness (Bousquet et al., 2000). Thus, it is highly probable that adaptive mechanisms have evolved permitting cells to promote inflammation, while simultaneously inducing counter-regulatory pathways to resolve potential chronic inflammation and to minimise inflammation-related trauma to resident structural cells. Airway PARs, especially PARs localised in the bronchial epithelium, may participate in this process by stimulating the release of various inflammatory chemokines and tissue remodelling mediators (Vliagoftis et al., 2000, 2001), while concurrently inducing the secretion of cytoprotective mediators in resident cells to reduce subsequent inflammation-induced damage.

One possible cytoprotectant mediated by PARs in the airway is prostaglandin (PG) E_2 (Lan et al., 2001). Endogenous PGE $_2$ conferred bronchoprotection against spasmogenic agents (Pavord & Tattersfield, 1995), and inhaled PGE $_2$ inhibited both the early and late response to allergen-induced bronchial reactivity (Pavord et al., 1993). PAR-stimulated PGE $_2$ release from the airways was generated from epithelial-derived COX-2 (Lan et al., 2001) (see Fig. 2), the inducible COX isoform that had been hypothesised to be up-regulated during cellular trauma and serve as a braking mechanism against perpetual airway inflammation and remodelling (Belvisi et al., 1998; Lazzeri et al., 2001; Pang, 2001). The finding that COX inhibitors induce airways hyperresponsiveness in allergic animals and a subset of asthmatic patients (aspirin-sensitive asthmatics) highlights the importance of this enzyme and its metabolites in regulating airway homeostasis (Babu & Salvi, 2000; Peebles et al., 2000; Szczeklik et al., 2000).

NO and CGRP have also been reported to possess potential bronchoprotective roles in the airways (Cadieux et al., 1999; Di Maria et al., 2000), and PAR-mediated cytoprotection has been demonstrated to occur through the release of these mediators in other tissue systems (Kawabata

et al., 1999a, 2001a; Vergnolle et al., 1999b; Fiorucci et al., 2001). However, PAR-mediated responses in several airway tissues occurred through NOS-insensitive pathway(s) (Cocks et al., 1999a; Chow et al., 2000; Schmidlin et al., 2001b), suggesting a minimal involvement of this mediator in PAR-mediated bronchoprotection.

If airway PARs regulate both pro-inflammatory and cytoprotective pathways, one can speculate that the use of PAR antagonists to resolve chronic airway inflammation may be unfavourably compromised by the suppressed synthesis of cytoprotective mediators, resulting in potential cellular toxicity and tissue dysfunction. Nevertheless, our current understanding of the relative contribution of PARs to pro-inflammatory and cytoprotective processes during airway disease is incomplete. Future investigations are likely to include examining the effects of PARs in animal models of airway oedema and inflammation utilising appropriate PAR activators/inactivators and PAR antagonists; examining the regulation of PARs in healthy and diseased bronchial tissue; as well as investigating the significance of PAR polymorphisms in airway disease. Such work would provide a clearer overview for the role of PARs in the airway during homeostasis and the diseased state, and a better understanding of airway PARs when assessing their potential as therapeutic targets.

11. Conclusion

In summary, PARs play an important role in modulating homeostasis and inflammation in the airways. Activation of PARs in the airway is likely to be a highly regulated process and dependent upon many factors, including the level of PAR expression, the relative presence of PAR activation/inactivating proteases, and the bioavailability of endogenous protease inhibitors in the extracellular space. Stimulating airway PARs triggers cellular growth and repair in the lungs, and may promote recruitment of inflammatory cells into the respiratory tract. Concurrently, PAR activation may also stimulate the release of bronchoprotective mediators such as PGE $_2$ as a cytoprotectant and a negative regulator of inflammation-induced injury (Table 3). However, the role of PARs on structural cells during airway diseases and the effects of PAR agonist peptides on activated inflammatory cells remain unknown and await future characterisation. Future findings will more clearly define the role of PARs in pathophysiology and its potential as a target for therapeutic intervention in airway disease.

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