

Role of protease-activated receptors in inflammatory responses, innate and adaptive immunity

V. Shpacovitch,^{*,1} M. Feld,^{*} M. D. Hollenberg,[†] T. A. Luger,^{*} and M. Steinhoff^{*}

^{*}Department of Dermatology and Ludwig Boltzmann Institute for Cell Biology of the Skin, University of Münster, Münster, Germany; and [†]Inflammation Research Network, Department of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada

Abstract: Serine proteases are well known as enzymes involved in digestion of dietary proteins, blood coagulation, and homeostasis. Only recent groundbreaking studies revealed a novel role of serine proteases as signaling molecules acting via protease-activated receptors (PARs). Important effects of PAR activation on leukocyte motility, cytokine production, adhesion molecule expression, and a variety of other physiological or pathophysiological functions have been described in vitro and in vivo. The crucial role of PAR activation during disease progression was revealed in animal models of different gastrointestinal pathologies, neuroinflammatory and neurodegenerative processes, skin, joint and airway inflammation, or allergic responses. This review focuses on the findings related to the impact of PAR deficiency in animal models of inflammatory and allergic diseases. Additionally, we observe the role of PAR activation in the regulation of functional responses of innate and adaptive immune cells in vitro. Understanding the mechanisms by which PARs exert the effects of serine proteases on immune cells may lead to new therapeutic strategies in inflammation, immune defense, and allergy. *J. Leukoc. Biol.* 83: 000–000; 2008.

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INTRODUCTION

Innate and adaptive immunity represents two main branches of host response to infection. Innate immune responses are classically considered as the first line of host defense, which serves to limit infection rapidly, soon after microorganism invasion. Innate immunity includes evolutionarily ancient mechanisms of host defense, rapid and nonspecific responses to infection [1]. In contrast, more antigen-specific and slowly initiating responses characterize adaptive immunity [2, 3]. Nonetheless, only a productive and integrated interaction between the innate and adaptive immune systems could establish successful and efficient host defense against invading pathogens [4].

The fact that ~1200 genes (4.5% of all human genes) encode proteases in the human genome reflects the importance of proteases in the human body under physiological and patho-

physiological conditions. However, the role of protease signaling in innate and adaptive immunity is just at the beginning of intensive investigation. Although, there are a number of ways that proteases can trigger cell signaling, a recently described family of protease-activated receptors (PARs) can account for a significant proportion of signaling generated by proteolytic enzymes (reviewed in refs. [5, 6]). This review will focus on PARs and their importance for the function of immune cells in vitro as well as for immune responses in vivo.

The unique mechanism whereby serine proteases signal via the PARs involves the cleavage of the receptor N-terminal part to expose a new, previously cryptic sequence. The exposed sequence remains tethered to the receptor and acts further as a receptor-activating ligand, named “tethered ligand” (reviewed in refs. [5, 6]). Some proteases could cleave PARs downstream of the tethered ligand sequence, making further proteolytic activation of PARs impossible (receptor inactivation; see Table 1). Thus, proteases can regulate PAR signaling by activation or inactivation. Four PARs have been cloned and characterized [5, 6]. PARs 1, 3, and 4 were the first identified targets for thrombin but can also be activated by trypsin or cathepsin-G (CG). In contrast, PAR₂ is resistant to thrombin but can be activated by trypsin, mast cell tryptase, leukocyte proteinase-3 (PR3), and bacteria-derived enzymes (see Table 1) [5–7]. PARs also can be activated without the need for proteolysis by synthetic peptides (so-called PAR-APs). PAR-APs have sequences based on those of the revealed tethered ligands [5, 6]. Specific PAR-APs are important probes for investigating the role of PAR activation, as serine proteases are known to cause PAR-dependent as well as independent responses in various cells. Although PARs 1, 2, and 4 signaling could be triggered upon activation by proteases or receptor-specific PAR-APs, the role of PAR₃, which cannot signal on its own, remains a bit of an enigma. Currently, PAR₃ is viewed as an accessory receptor for PAR₁ or PAR₄ [8–10].

More general information concerning PARs, leukocyte- and pathogen-derived proteases signaling via PARs, is provided in recent reviews [5–7]. This review describes the role of PAR

¹ Correspondence: Dept. of Dermatology, Laboratory of Cell Biology, University of Münster von-Esmarch-Str. 58, 48149 Münster, Germany. E-mail: shpacovi@ukmuenster.de

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TABLE 1. PARs and the Proteases Acting via PARs

	PAR ₁	PAR ₂	PAR ₃	PAR ₄
Tethered ligand sequence	SFLLRN (h) SFLLRN (r, m)	SLIGKV (h) SLIGRL (r, m)	TFRGAP (h) SFNGGP (m)	GYPGQV (h) GYPGKF (m) GFPGKP (r)
Available activating peptides	SFLLRN-NH ₂ (h, m, r; nonspecific) TFLLRN-NH ₂ (m, h, r)	SLIGRL-NH ₂ (m, r) SLIGKV-NH ₂ (h)	None known	GYPGQV-NH ₂ (h) GYPGKV-NH ₂ (m) AYPGKF-NH ₂ (r)
Available receptor antagonists or inhibitors ^a	Trans-cinnamoyl-parafluoro-Phe-Paraguanidino-Phe-Leu-Arg-Arg-NH ₂ (BMS-200261, m); Mercaptopropionyl 1-Phe-Cha-Arg-Lys-Pro-Lys-Pro-Asn-Asp-Lys-NH ₂ (r); SCH79797 (m, r); RWJ56110; RWJ58259	ENMD-1068 (produced by EntreMed, m)	None known	Trans-cinnamoyl-YPGKF-NH ₂ (m, r); Pepducin antagonist (P4pal-il)
Classical activating proteases of mouse and human PARs	Thrombin	Trypsin; mast cell tryptase	(Thrombin) ^b	Thrombin, Trypsin
Immune cell-derived activating proteases ^c	Cathepsin G (h); Granzyme A (h, m)	Granzyme A (h)	None known	Cathepsin G (h)
Proteases that could activate or inactivate PARs on human cells	Leukocyte elastase	Cathepsin G; Leukocyte elastase Proteinase 3	None known	None known
Pathogen-derived activating proteases ^c	Gingipains RgpB and HRgp A (h)	Der p 3, Der p 9 Der p 1 (h); Gingipain-R (h, m); Thermolysin (h); Serralysin (h)	None known	Gingipain RgpB and HRgpA (h)

Four different PARs have been detected on immune cells. Thrombin, trypsin, and tryptase are considered as classical PAR activators. Moreover, pathogen- and immune cell-derived proteases seem to activate or inactivate PARs depending on the position of an enzymatic cleavage site in the N-terminal domain. The synthesis of PAR activating peptides (PAR-APs) was based on the tethered ligand sequence. However, later, several modified, activating peptides with furoyl- and trans-cinnamoyl groups became available (tc-LIGRLO-NH₂; 2-furoyl-LIGKV-OH, for example). m, Mouse; r, rat; h, human. ^a If PAR antagonist was used not only in experiments with human cells but also in animal experiments, the species are indicated in parentheses according to abbreviations given above. ^b (Thrombin). Thrombin was demonstrated to cleave PAR₃ without induction of intracellular Ca²⁺ changes. ^c The ability of protease to activate PARs expressed on human and/or rodent cells is indicated in parentheses according to abbreviations. SFLLR-NH₂ and related peptides are proved to be nonspecific PAR₁/PAR₂ activators.

activation in the function of innate and adaptive immune cells in vitro and focuses on the role of PAR deficiency for the development of different inflammatory pathologies in animal models.

PARs AND THE FUNCTION OF IMMUNE CELLS IN VITRO

Figures 1 and 2 illustrate the effects of PAR₁ and PAR₂ activation on the function of human immune cells. The mechanism of PAR activation is also displayed in these schemes. In the following sections, more detailed information is summarized concerning the role of PAR activation in the regulation of functional responses of human and nonhuman immune cells.

Polymorphonuclear granulocytes

Neutrophils

The expression of functional PAR₂ on the neutrophil cell surface varies among different human donors. This variability has been confirmed by FACS analysis and an intracellular calcium ([Ca²⁺]_i) response induced after treatment of neutrophils with PAR₂ agonists [11–13]. Human neutrophils also

express PAR₃ mRNA, but the functional role of this receptor, except to act as a coreceptor for PAR₁, is not clear [14]. Although human neutrophils isolated from male or nonpregnant female, healthy volunteers do not express PAR₁ [14, 15], Wang and colleagues [16] have reported that neutrophils obtained from donors with normal or preclamptic pregnancies do express PAR₁ mRNA. The functionality of this receptor remains to be verified.

The expression of functional PAR₂ by human neutrophils was first described by Howells and colleagues [11], who showed that the PAR₂-AP, SLIGKV, increased [Ca²⁺]_i levels and induced neutrophil shape changes. The authors also demonstrated that costimulation of isolated human neutrophils with the PAR₂-AP, along with the well-known neutrophil activator fMLP, leads to a more pronounceable up-regulation of Mac-1 (CD11b/CD18) expression than does fMLP alone. In further studies, Shpacovitch and colleagues [13] demonstrated that stimulation of human neutrophils with PAR₂ agonists (tc-LIGRLO-NH₂ and trypsin) led to a significantly enhanced motility of these cells in three-dimensional (3-D) collagen lattices, suggesting a role of PAR₂ in the regulation of neutrophil migration through the extracellular matrix (ECM) toward the site of inflammation and/or infection. Moreover, PAR₂

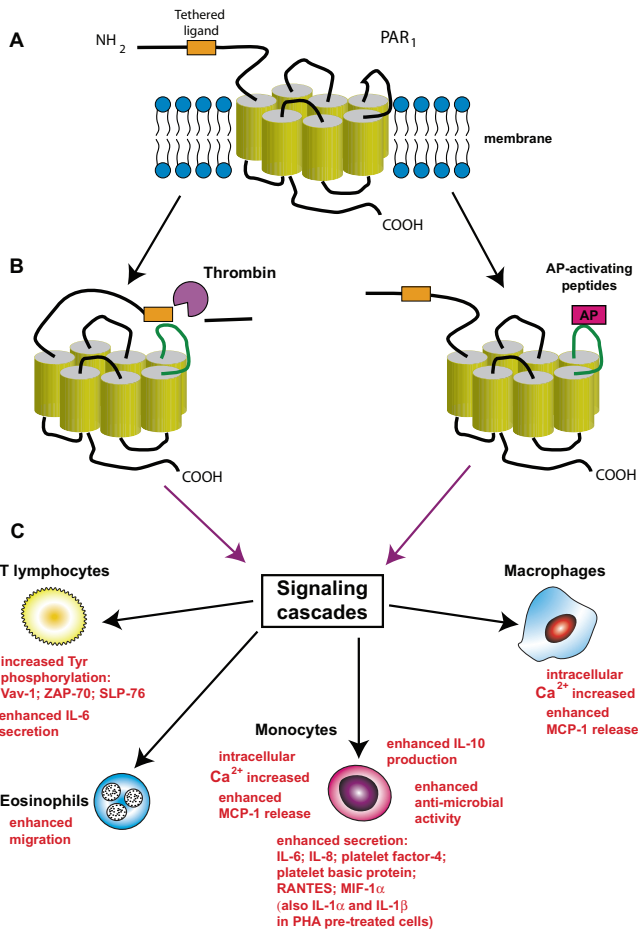


Fig. 1. Scheme of the activation of PAR₁ and PAR₁-triggered responses in human immune cells. (A) The inactive status of PAR₁ is associated with a hidden N-terminal-tethered ligand sequence. The tethered ligand part cannot interact with the second extracellular loop of the receptor and does not activate it. (B) The accessible serine protease cuts the N-terminal part of the receptor and unmasks a tethered ligand sequence of the receptor. Downstream cleavage of the receptor results in the receptor inactivation. Further, the tethered ligand interacts with the second extracellular loop of the same receptor and thus, triggers signaling events. Synthetic activating peptides (PAR₁-APs) activate PAR₁ without proteolytic cleavage. PAR₁-AP directly interacts with the second extracellular loop of the receptor, inducing identical signaling pathways as the natural protease. (C) PAR₁ activation on human immune cells leads to various responses mentioned on the scheme. ZAP-70, ζ-associated protein 70; SLP-76, Src homology 2 domain-containing lymphocytic protein of 76 kDa; MIF-1α, macrophage migration inhibitory factor-1α.

agonists enhance the shedding of L-selectin from the cell surface of human neutrophils. The effect of PAR₂ agonists at neutrophil motility in 3-D collagen lattices was significantly abolished by the L-selectin shedding inhibitor KD-IX-73-4 [13]. This finding indicates a link between PAR₂ agonist-induced shedding of L-selectin and the enhanced motility of neutrophils through the ECM. Further, it was reported that simultaneous stimulation of human endothelial cells and neutrophils with proteases (trypsin, tryptase) or PAR₂-tcAP reduces transendothelial migration of granulocytes and also prolongs neutrophil survival in vitro [17]. Additionally, PAR₂ agonists have been reported to enhance the expression of Mac-1 and VLA-4 on neutrophils [13] and stimulate the release of IL-1β, IL-8, and IL-6 from these cells [13]. Recently,

tryptase, trypsin, and PAR₂-APs (SLIGKV-NH₂ or tc-LI-GRLO-NH₂) were demonstrated to induce lactoferrin secretion by human neutrophils [18].

It is also important to note that neutrophil granule serine proteases such as human leukocyte elastase, CG, and PR3 are able to activate or disarm PARs (reviewed in ref. [7]).

In contrast to human cells, the expression of PAR₄ has been detected immunohistochemically on the cell surface of rat neutrophils [19]. In further studies, it was revealed that local injection of PAR₄-AP (AYPGKF-NH₂) induced neutrophil recruitment and edema formation in a rodent paw inflammation model [20]. Additionally, application of a PAR₄ antagonist (P4pal-10) attenuated neutrophilic, inflammatory responses in a mouse model of disseminated intravascular coagulation [21].

The expression pattern of PARs on neutrophils is different in rodents (mice, rats) and in humans. PAR₄ activation appears to

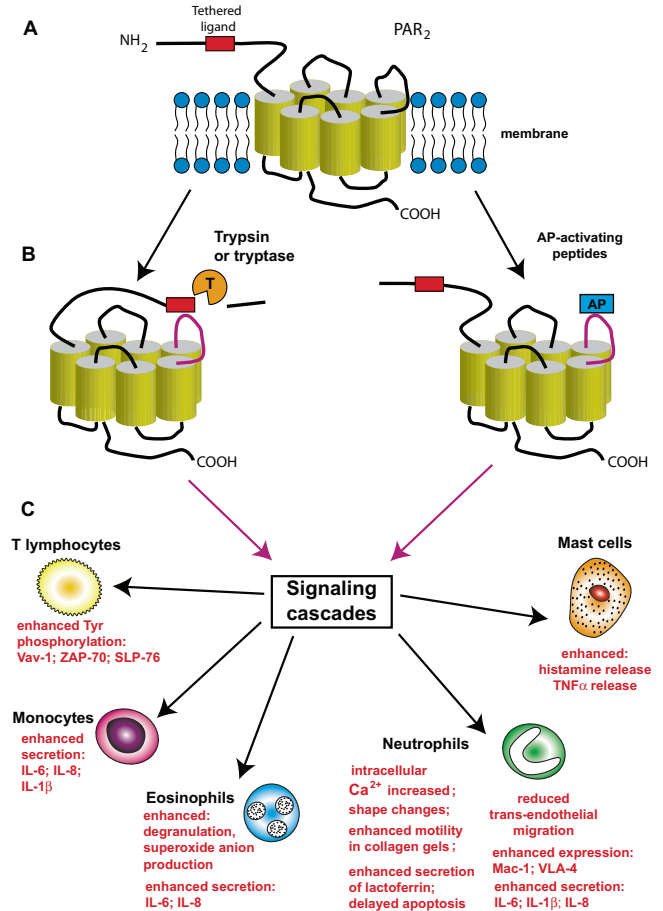


Fig. 2. Scheme of the activation of PAR₂ and PAR₂-induced effects in human immune cells. (A) Inactive PAR₂ has an N-terminal-tethered ligand sequence, which remains cryptic. This tethered ligand does not interact with the second extracellular loop of the receptor and does not trigger downstream signaling events. (B) Endogenous or exogenous serine protease could cut the N-terminal part of the receptor, unmasking a previously cryptic tethered ligand sequence of the receptor. Further, the tethered ligand interacts with the second extracellular loop of the same receptor triggering signaling events. Synthetic activating peptides (PAR₂-APs) activate PAR₂ without proteolytic cleavage. PAR₂-AP directly interacts with the second extracellular loop of the receptor, inducing identical signaling pathways as the natural protease. (C) PAR₂ activation on human immune cells results in various immune cell responses. Mac-1, Membrane-activated complex 1.

affect the migratory ability of rodent neutrophils. However, PAR₂ seems to be functionally the most important member of the PAR family expressed by human granulocytes. These facts limit the use of mouse models for the investigation of the PAR-mediated responses on neutrophil functions. Despite well-documented effects of PAR₂ agonists at cytokine production, motility, and adhesion molecule expression by human neutrophils, effects of PAR₂ activation at main functions of human neutrophils—phagocytosis and killing of bacteria—remain unknown. Moreover, further investigation of the PAR-associated effects of neutrophil granule proteases at functions of epithelial, endothelial, and immune cells also looks prospective. Such studies might reveal new approaches that allow direct interfering of the inflammatory and immune responses.

Eosinophils and basophils

Human basophils do not possess any member of PAR's family [22]. There is no report indicating that rodent basophils possess any PARs. However, human eosinophils reportedly express PAR₂ mRNA and protein [14, 15]. Receptor expression has been detected on the surface of eosinophils as well as intracellularly [14, 15]. Moreover, the number of PAR₂-positive eosinophils increases during seasonal allergic rhinitis in the nasal mucosa [23]. The ability of human eosinophils to express functional PAR₁ remains unclear. Miike and colleagues [14] failed to observe PAR₁ mRNA expression and functional responses to PAR₁ agonists in isolated human eosinophils. Nevertheless, Bolton and colleagues [15] found that PAR₁ is expressed in isolated human eosinophils, but this group did not find significant functional responses in eosinophils after PAR₁ agonist stimulation. However, more recent studies have been able to demonstrate effects of thrombin and a specific PAR₁-AP on eosinophil migration [24]. The different outcomes for these studies could be explained by the variations in experimental procedures and a possible pre-activation of eosinophils during preparation followed by receptor internalization or inactivation. Thus, the presence and functionality of PAR₁ on human eosinophils merit a further in-depth evaluation.

In contrast with the information summarized above, PAR₂, despite its low and variable eosinophil surface expression amongst donors, seems to be the predominant functional member of the PAR family on these cells in humans. Indeed, trypsin and a PAR₂-AP (SLIGKV-NH₂) can induce superoxide anion production and degranulation of human eosinophils via PAR₂ [14, 15]. This effect of trypsin requires its proteolytic activity, supporting the idea that the responses are associated with PAR₂ activation on human eosinophils [14].

The data concerning the influence of PAR₂ deficiency on OVA-induced airway eosinophilia in mice remain contradictory. Takizawa and colleagues [25] reported that the number of eosinophils is reduced significantly in bronchoalveolar lavage fluid (BALF) of PAR₂ knockout mice as compared with wild-type animals after OVA challenge. In contrast, De Campo and Henry [26] demonstrated that intranasal administration of PAR₂-AP at the time of OVA challenge also causes a reduction in the numbers of BALF eosinophils.

Findings concerning the role of PAR₂ in eosinophil activation are important, as serine and cysteine proteases produced

by allergenic organisms such as fungi, mites, and pollens might act in part by triggering PAR₂ signaling. Additionally, during the allergic response and subsequent mast cell degranulation, the released tryptase and chymase may also stimulate PAR₂ on human eosinophils. Several mite-derived proteases (Der p 3, Der p 9, and Der p 1) are capable of regulating PARs, including PAR₂ [27, 28]. However, whether mite-, fungus-, or pollen-derived proteinases activate PAR₂ expressed on eosinophils remains unknown. Moreover, mite-derived proteases are reported to mediate their effects also via a PAR₂-independent mechanism [29]. The biological and pathophysiological impact of these activation mechanisms thus need to be clarified in the future.

Mast cell tryptase is thought to induce some of its effects via activation of PAR₂ [30–33]. However, the ability of this enzyme to activate PAR₂ expressed on eosinophils needs further investigation. Temkin and colleagues [34] demonstrated that stimulation of isolated human eosinophils with human recombinant as well as with a human mast cell line 1 (HMC-1)-derived tryptase results in enhanced release of IL-6 and IL-8 by eosinophils [34]. These tryptase-induced effects on cytokine release by human eosinophils were reduced by adding an anti-PAR₂ antibody that can block receptor cleavage/activation. On the basis of these findings, the authors suggested that the effects of tryptase on eosinophil functions may be at least partially mediated via PAR₂ activation [34]. However, Vliagotis and colleagues [35] showed that induction by mast-cell tryptase of eosinophil peroxidase (EPO) and β -hexosaminidase (β -hex) release from human eosinophils does not depend on PAR₂ activation. The authors based their conclusion on the inability of the PAR₂-AP (SLIGRL-NH₂) to induce the release of EPO and β -hex from eosinophils. Thus, some tryptase effects on eosinophils may be PAR-independent.

In summary, the ability of allergen-derived proteases (house dust mite, cockroach, and others) to mediate their effects on functions of human eosinophils via PARs remains enigmatic and needs further investigation. New findings in this field might be useful, not only for a better understanding of the way of protease signaling to eosinophils but also could serve for designing novel, anti-allergic treatment strategies in humans.

Monocytes, macrophages, and dendritic cells (DC)

Monocytes

According to the work of Colognato and colleagues [36], isolated human monocytes express PAR₁, PAR₂, and PAR₃ mRNA and at protein levels PAR₁ and PAR₃. A receptor-selective PAR₁-AP as well as thrombin were demonstrated to stimulate an increase of [Ca²⁺]_i levels in human monocytes. Any role for PAR₃ in monocytes remains uncertain, as this receptor does not signal on its own. That said, evidence for the ability of undifferentiated human monocytes to express functional PAR₂ appears to be conflicting. Colognato and colleagues [36] reported that human monocytes do not express PAR₂ on the cell surface, whereas a report by Johansson et al. [37] demonstrated that human monocytes display functional PAR₂. Colognato's group [36] used monocytes separated mainly from buffy coats by a surface adherence protocol. They used an affinity bead (anti-CD14 MACS microbeads; positive

selection method) protocol only for monocyte isolation in RT-PCR experiments. Johansson and colleagues [37] used human monocytes isolated from healthy adult volunteers via blood collection in a heparinized tube, followed by negative magnetic cell sorting (MACS method). Isolation of human monocytes by adhesion might affect the monocyte phenotype [38], and thus, the differences between the apparently conflicting results related to the presence of PAR₂ might be a result of differences in the methods of cell isolation [37]. Moreover, the intracellular stores of PAR₂ were found in human monocytes. These stores could be mobilized rapidly to the monocyte cell surface [37]. Thus, the ability of human monocytes to express functional PAR₂ may also depend on the state of cell activation.

Stimulation of isolated monocytes with thrombin as well as with a receptor-selective PAR₁-AP (TFLLRNPNDK) leads to the up-regulation of MCP-1 expression and its release from human monocytes. This effect of thrombin was attenuated by the PAR₁-blocking antibody WEDE15, which inhibits proteolytic activation of PAR₁ [36]. The anti-apoptotic effect of plasminogen treatment demonstrated on isolated human monocytes and U937 cells (a human monocyte cell line) appears to depend on PAR₁ activation, as anti-PAR₁-blocking antibodies diminished this effect [39]. Thrombin as well as a PAR₁-AP can modulate the production and release by monocytes of IL-6, platelet basic protein, and platelet factor 4 and can also stimulate the proliferation of U937 cells [40–44]. Additionally, treatment of human monocytes with thrombin or the nonselective PAR₁/PAR₂-AP (SFLLRNPNDKYEPF; named SFLLRN-14) enhances the ability of these cells to kill bacteria such as *Salmonella enterica* serovar typhimurium or *Listeria monocytogenes* [45]. Unfortunately, in all of these studies [40–45] SFLLRN-14, SFLLR-NH₂, or SFLLR-NH₂ was used as a PAR agonist; however, they can activate PARs 1 and 2 [46, 47]. Thus, although PAR₁ would appear to be involved, based on the results with thrombin, which does not readily activate PAR₂, a cooperative role for PAR₂ along with PAR₁ in the monocytes cannot yet be ruled out. Further work with the PAR₁- and PAR₂-selective agonists TFLLR-NH₂ and SLIGKV-NH₂ should be able to resolve this issue.

Monocyte-derived and residential macrophages

Human monocyte-derived macrophages express PAR₁, PAR₂, and PAR₃ at the mRNA and protein levels [36]. Stimulation of monocyte-derived macrophages with PAR₁- or PAR₂-selective APs (TFLLRNPNDK or SLIGKV-NH₂, respectively) induces an increase in [Ca²⁺]_i level, confirming that both receptors are functional [36]. Prolonged treatment with IL-4 significantly reduces the expression of PAR₁, PAR₂, and PAR₃ by monocyte-derived macrophages [36]. PARs are reported to be expressed by different types of human tissue macrophages. Human alveolar macrophages express PAR₁ and PAR₂. The level of PAR₁ protein expression has been found to be higher in smokers than in healthy nonsmokers, but the expression levels of both receptors in asthmatic patients were comparable with those in healthy humans [48]. Immunohistochemical staining has demonstrated the expression of PAR₂ on vascular macrophage-derived foam cells, indicating a potential role of this receptor in the development of atherosclerotic lesions [49]. PAR₄ expression could not be verified in monocyte-derived

macrophages [36]. However, studies in the human liver (normal, cirrhotic, or hepatocellular carcinomas) revealed PAR₄ expression to be restricted to macrophage-like cells (Kupfer cells), B lymphocytes, and nerves [50].

Stimulation of human monocyte-derived macrophages with thrombin or specific PAR₁-AP (TFLLRNPNDK) results in up-regulation of the expression and release of MCP-1 by these cells. PAR₂-AP failed to induce those effects in human monocyte-derived macrophages [36].

It is also interesting to notice, that murine brain macrophages (microglia cells) express mRNA for all PARs at different levels (the lowest for PAR₄) [51]. Activation of mouse microglial PAR₁ induces [Ca²⁺]_i increase and transient activation of p38 as well as p44/42 MAPKs [52]. Moreover, PAR₁ appears to contribute directly to thrombin-induced microglial proliferation in mice [52]. On the other hand, the proteolytic activity of thrombin was unlikely involved in the protease-mediated effects on chemokine and cytokine production in murine microglia cells [53]. When tested, the ability of thrombin to activate rat microglial cells, as assessed by NO release, was found not to be mimicked by a nonselective PAR₁/PAR₂-AP and therefore, appeared to be PAR₁-independent [54]. However, a role for thrombin-activated PAR₄ in that study was not explored.

DC

Human monocyte-derived DC, generated after stimulation of monocytes with GM-CSF and IL-4, express PAR₁, PAR₂, and PAR₃ mRNA but do not express the PARs at the protein level [36]. The lack of PAR₂ expression was also reported for human blood DC derived from CD34-positive stem cells [37]. However, thrombin is able to induce the release of MCP-1, IL-10, and IL-12 from plasmacytoid and myeloid DC isolated from human blood. This effect of enzyme might be mediated via PAR₁, as PAR₁ is expressed by plasmacytoid and myeloid DC [55].

Activation of PAR₂ with a receptor-selective AP (SLIGRL-NH₂) can stimulate mouse DC development [56]. Additionally, bone marrow progenitor cells from PAR₂ knockout mice failed to generate DC under standard culture conditions (GM-CSF and IL-4 stimulation) but required additional stimulation by TNF-α [56]. Further, Csernok and colleagues [57] demonstrated that PR3, a well-known “Wegener autoantigen”, also induces differentiation of DC via a PAR₂-dependent pathway.

DC isolated from the mouse spleen and bone marrow progenitor cells express the identical pattern of PARs [56]. The level of PAR₁ and PAR₂ expression significantly decreases in bone marrow progenitor cells during cell culture and also remains low in mature spleen DC [56]. The work of Colognato and colleagues [36] sheds light on the possible mechanism of such regulation in vitro, wherein prolonged treatment of cultured monocytes with IL-4 resulted in a significant reduction of PAR expression during DC generation.

Despite that the role of PARs in functional responses of brain macrophages (microglial cells) is well documented, the impact of PAR activation at functions of other macrophages, especially in disease models, is still poorly investigated in rodents. Human monocytes, macrophages, and DC possess at least one functional member of the PAR family, namely PAR₁.

However, the role of protease signaling mediated via PAR₁ in main functions of these cells, such as antigen uptake and processing, phagocytosis, and the production of inflammatory mediators, needs further investigation.

Mast cells

HMCs are well recognized as key players in the initiation of allergic diseases and also as participants in the host response to certain types of parasitic infections. However, little is known about the impact of PARs and their proteolytic activators on mast cell function. Mast cells located in human tonsils, skin, and colon express PAR₂ [58–60]. Trypsin as well as PAR₂-AP (SLIGKV) stimulation results in a concentration-dependent release of histamine from HMCs in the skin and tonsils [59, 60]. Additionally, skin HMCs have been found to express mRNA for PAR₁, PAR₃, and PAR₄ [60]. HMCs from different tissues display PAR₂- and PAR₁-positive intracellular staining on tryptase-containing granules [61]. Leukemia-derived HMC-1 possesses functional PAR₂ and PAR₄ and also expresses mRNA for PAR₁ and PAR₃ [60, 62]. Stimulation of HMC-1 with PAR₂- or PAR₄-APs enhanced TNF- α secretion by these cells [62].

Murine mast cells respond to PAR₁ activation with enhanced IL-6 secretion [63]. Moreover, thrombin and a receptor-selective PAR₁-AP have been found to induce the adhesion of murine bone marrow-derived mast cells to fibronectin and laminin. This process was found to involve α 4 and α 5 integrins along with PI-3K, MAPK, and protein kinase-3 signaling pathways [64]. Stimulation with IL-12 down-regulates the expression of PAR₂ and enhances the expression of PAR₄ on murine mast cell line P815 cells [65]. Rat peritoneal mast cells (PMCs) express PAR₁ and PAR₂ mRNA [66, 67]. However, the functional role for these receptors on PMCs still remains unclear. On the one hand, stimulation with PAR₁- or PAR₂-AP of PMCs does not appear to trigger degranulation [66]. On the other hand, the nonselective PAR₁/PAR₂-AP (SFFLRN) is capable of activating NO [68] as well as β -hex and histamine release from PMCs [69]. Further studies are, however, demanded to clarify the precise role of PARs in mast cell function and in mast cell-mediated hypersensitivity.

Mast cell tryptase has been proposed as an important regulator of PAR₂ (reviewed in refs. [7, 70]). This enzyme is reported to activate PAR₂ on different cell types such as lung fibroblasts [71], smooth muscle cells [72], myenteric neurons [31], keratinocytes [32, 73], endothelial cells [74, 75], testicular peritubular cells [76], and primary HMCs [60]. However, the ability of mast cell tryptase to activate PAR₂ in vivo under physiological and pathophysiological conditions needs further investigation. Finally, future studies should verify a potential role of PAR antagonists for the treatment of inflammatory, parasitic, and allergic diseases in which mast cells are involved.

B and T lymphocytes

B cells

Cell surface expression of PAR₄ has been detected on B cells in the human liver [50], but the role of this receptor in B cell function remains unknown. In mice, activation of PAR₂ stimulates B lymphocyte adhesion [77].

T cells

Human T cell lines and human T cells in tissues appear to express PAR₁, PAR₂, and PAR₃ [78–81]. Stimulation of peripheral blood lymphocytes with PHA and PMA enhances the level of PAR₂ mRNA expression in these cells [78]. Mouse T lymphocytes also express functional PAR₂, although its biology remains to be clarified [82].

Stimulation of human Jurkat T cells with thrombin, trypsin, or PAR₁- or PAR₂-APs has been found to elevate $[Ca^{2+}]_i$ [78]. Further, Bar-Shavit and colleagues [79] found that the activation of PARs (PAR₁, PAR₂) in Jurkat T cells induces tyrosine phosphorylation of Vav-1 and also leads to tyrosine phosphorylation of ZAP-70 and SLP-76, which are known to play a crucial role in TCR signaling. Unfortunately, these investigators used a PAR₁/PAR₂ nonselective agonist (SFLLRNPNDK). Therefore, the observed effects might have been a result of a simultaneous activation of PAR₁ and PAR₂. Although a functional role for PAR₃ on T cells was suggested in that study, further work revealed that the PAR₃-AP (TFRGAPPNSF) does not activate PAR₃ in Jurkat T cells but rather stimulates PAR₁ or PAR₂ [80]. In human primary T cells, treatment with thrombin, trypsin, tryptase, and PAR-APs resulted in an increased IL-6 secretion [81]. However, the analysis of the underlying signaling cascades and their role under physiological and pathophysiological conditions need further investigation. Recently, an important role for PAR₂ signaling has been found for the cytokine production by mouse CD4⁺ T cells [82]. A reduction of IL-4 production by splenic CD4⁺ T cells was found during OVA-induced airway inflammation in PAR₂-deficient mice, as compared with wild-type animals. After antigen stimulation, IFN- γ production was enhanced in PAR₂ null animals as compared with wild-type mice. This PAR₂-mediated regulation of T cell cytokine production appears to be associated with JNK1 phosphorylation [82].

The role of PAR₂ in cytokine production and other responses of human and murine T cells has been shown. However, there is a lack of detailed information concerning the involvement of PAR₁ in the regulation of T cell function and the role of PARs in T cell-mediated diseases.

PAR-MEDIATED EFFECTS ON LEUKOCYTES IN VIVO

Estimating a potential effect of PAR activation on leukocyte trafficking, Zimmerman and colleagues [83] used a nonselective PAR₁/PAR₂ agonist (SFLLRNPNDKYEPF). In a subsequent work, using a similar method of rat mesenteric venule superfusion in vivo, but with PAR-selective peptide agonists, Vergnolle and colleagues [19] further investigated the role of different thrombin receptors (PAR₁ and PAR₄) in leukocyte rolling and adherence. Topical application of the selective PAR₁ agonist (TFLLR-NH₂) to rat mesenteric venules failed to reproduce the increased leukocyte rolling and adhesion observed after topical thrombin application. Moreover, topical addition of PAR₂-APs in the rat preparation resulted in increased leukocyte rolling and adhesion [84]. Taken together, these findings suggest that the effects observed previously [83]

were predominantly a result of PAR₂ activation. The role of PAR₂ in the regulation of leukocyte motility in vivo was also investigated in further studies using *PAR₂* null mice. In comparison with wild-type animals, PAR₂-deficient mice showed significantly decreased leukocyte rolling in a model of acute inflammation induced by surgical trauma [85]. Additionally, an increase in lymphocyte adhesion and reactive oxygen species (ROS) production in response to trypsin stimulation was abolished in *PAR₂* knockout mice, indicating a PAR₂-dependent mechanism for these trypsin-induced effects [77].

The effect of intranasal administration of proteases and PAR-APs on leukocyte recruitment into the airways has been analyzed in a murine model. Thrombin-induced recruitment of polymorphonuclear cells appeared to be associated with PAR₄ activation, as the inhalation of a nonselective PAR₁/PAR₂-AP failed to reproduce thrombin-induced effects, and thrombin itself did not effectively activate PAR₂ [86]. Additionally, a thrombin-induced macrophage adhesion in vivo has been demonstrated to be PAR₁-independent [87]. In summary, PAR₂ as well as PAR₄ can be considered as the main members of the PAR family affecting leukocyte motility in vivo.

Role of PARs in inflammatory disease models

A significant role of PAR activation in the progression of inflammatory diseases has been demonstrated in studies that used different animal models, including transgenic and knockout mice [88–93]. However, only a few of these studies analyzed the involvement of PAR agonist-stimulated immune cells in disease progression. Generally, it is important to notice that the disruption of *PAR₁* and *PAR₂* genes might benefit or worsen the prognosis for the host depending on the type and stage of the inflammatory disease [91, 94–96]. The effect of PAR₄ deficiency on the development of inflammatory diseases is still poorly investigated. Additionally, the downstream signaling cascades triggered after PAR activation during inflammation in vivo are just at the beginning of investigation [93]. The effects of PAR deficiency in animal models of different inflammatory pathologies are presented in **Table 2**. The following review section provides detailed information concerning important types of inflammatory animal models, which were investigated.

PARs and endotoxemia models

Sepsis represents a systemic host response to bacterial infection. Sepsis is known to activate coagulation and inflammation [104]. Remarkably, activated protein C (APC), an endogenous anticoagulant that is activated by thrombin, is protective in sepsis [105, 106]. On the other hand, PARs are known to be activated by coagulation proteases (thrombin; TF-FVIIa and TF-FVIIa-FXa complexes; factor FXa; reviewed in ref. [107]), indicating the potential way by which coagulation might affect inflammation [108]. Moreover, PAR₁ seems to be the target for endothelial protein C receptor-dependent APC signaling [109]. Altogether, these studies served as a basis for further investigation into the effect of *PAR* gene disruption at the progression of sepsis in animal models of this disease.

Pawlinski and colleagues [110] demonstrated that the deficiency of PAR₁ or PAR₂ alone did not affect mouse survival in a model of endotoxemia. The lack of any significant effect of the PAR₂ deficiency at the pathophysiological changes in-

duced by LPS in the mouse was also confirmed in the work of Kazerani and coauthors [111]. However, an application of hirudin (a thrombin inhibitor) in *PAR₂* knockout mice protected these animals better than *PAR₂* wild-type mice against LPS-induced lethality [110]. This fact allowed the authors to suggest that the combination of PAR₂ deficiency and inhibition of thrombin might be beneficial for mouse survival during LPS-induced endotoxemia.

This hypothesis was checked later in the study of Camerer and colleagues [112] about PAR single-deficient mice (*PAR₁*, *PAR₂*, and *PAR₄* knockouts) and on double-knockouts such as *PAR₁^{-/-}:PAR₂^{-/-}* and *PAR₂^{-/-}:PAR₄^{-/-}*. These authors also used i.p. injection of LPS to induce endotoxemia. The results of the study clearly indicated that neither single PAR deficiency nor combined PAR deficiency (such as *PAR₁^{-/-}:PAR₂^{-/-}* or *PAR₂^{-/-}:PAR₄^{-/-}*) played any significant role in mouse survival during LPS-induced endotoxemia [112]. Altogether, the results from a mouse model of endotoxemia do not support any role for PAR-mediated protease signaling in murine LPS-induced lethality.

However, it is still important to notice that a mouse model of LPS-induced endotoxemia might just hardly mimic the changes observed during human sepsis [113]. Thus, it cannot be considered as a really adequate model of human sepsis but rather as a model of endotoxic shock. Moreover, the circulating LPS levels in septic human patients are also reported to be low [113, 114]. According to several criteria, the cecal ligation and puncture model seems to be a more appropriate model, reflecting more precisely the dynamics of sepsis occurring in humans [113]. Unfortunately, the role of PAR deficiency for pathophysiological changes, which occur in this model, was not investigated.

PARs and inflammatory gastrointestinal diseases

PAR activation appears to play a significant role in gastrointestinal inflammation. Inflammatory bowel diseases (IBD) such as Crohn's disease, ulcerative colitis, and infectious colitis are frequent and potentially life-threatening inflammatory diseases. In the normal gut, PARs are constitutively expressed in the colon and small intestine by epithelial cells, colon mast cells, nerves, and smooth muscle cells [58, 115–119]. A role of PARs in IBD was confirmed in several in vitro and in vivo studies [120–122], some of which showed the involvement of particular immune cell types [91, 94].

PAR₂ activation reportedly played a protective role in a mouse model of TNBS-induced colitis (intrarectal administration of TNBS) [91]. T cell-derived cytokines (IL-2, IL-12, IFN- γ , TNF- α) were shown to be involved in the development of TNBS-induced colitis [123–125], which suggests that anti-inflammatory effects of PAR₂ activation may be associated with the modulation of T lymphocyte function in this tissue. Moreover, PAR₂-AP prevented the induction of Th₁ and Th₂ proinflammatory cytokines in the colon that was caused by TNBS injection. PAR₂ agonist stimulation in vivo affected T lymphocyte proliferation in the lamina propria after exposure to anti-CD3/CD28 [91]. These findings indicate a protective role of PAR₂ activation in a mouse model of a chronic, chemically induced colitis. However, PAR₂ activation seems to contribute in the development of enteritis caused by toxin A from *C.*

TABLE 2. The Crucial Effects of PAR Deficiency or PAR Agonist Stimulation in Animal Models of Inflammation

	Mouse model	Inflammatory effects of PAR deficiency	Stimulation/ antagonism	Reference
PAR ₁ ^(-/-)	TNBS- and DSS-induced colitis	attenuated colitis development	-/-	[94]
	bladder instillation of LPS, SP, and antigen	attenuated inflammatory response	-/-	[93]
	crescentic GN	reduced crescent formation and inflammatory cell infiltration	-/-	[97]
PAR ₂ ^(-/-)	BSA-induced arthritis	reduced antigen-specific Ig response and cytokine expression (IL-1 and -6)	-/-	[98]
	adjuvant monoarthritis	abolished chronic inflammatory response (joint swelling/damage)	-/-	[88]
	enteritis caused by toxin A from <i>Clostridium difficile</i>	reduced inflammatory response to toxin A	-/-	[99]
	experimental autoimmune encephalomyelitis	reduced T cell proliferation and expression of inflammatory genes in the CNS, reduced neuropathological changes	-/-	[100]
	Tat-induced neuroinflammation	severity of neuroinflammation and neuronal damage increases	-/-	[101]
	allergic contact dermatitis	attenuated inflammatory responses (swelling, plasma extravasation, leukocyte adherence)	-/-	[89]
	Type of stimulation or mouse model	Inflammatory effects of receptor stimulation or antagonism	Stimulation/ antagonism	Reference
PAR ₁ ^{wt}	intracolonic administration of PAR ₁ agonists in TNBS-treated mice (induced colitis)	enhanced inflammatory responses (macroscopic damage score and MPO activity)	TFLLR-NH ₂	[94]
	PAR ₁ antagonism in oxazolone-treated mice (colitis)	provoked oxazolone-mediated inflammation colitis	SCH79797	[95]
	bladder administration of PARs 1, 2, and 4 agonists	enhanced granulocyte infiltration in bladder	SFFLRN-NH ₂ SLIGRL-NH ₂ AYPGKF-NH ₂	[93]
	PAR ₁ antagonism in GN	reduced crescent formation and T cell as well as macrophage infiltration	Hirudin	[97]
PAR ₁ ^{wt} and PAR ₂ ^{wt}	influenza A virus infection model	elevated PAR ₁ and - ₂ levels in infected mice and recruitment of PAR-positive mononuclear cells	-/-	[102]
PAR ₂ ^{wt}	PAR ₂ activation in TNBS-induced colitis	provided protection against colon inflammation	SLIGRL-NH ₂	[91]
	intra-articular injection of PAR ₂ agonists in an adjuvant monoarthritis model	resulted in prolonged joint swelling and synovial hyperemia	SLIGRL-NH ₂ 2-furoyl-LIGKV-OH	[88]
	inhibition of PAR ₂ up-regulation carrageenan/kaolin-induced arthritis	diminished joint swelling and inflammatory response	siRNA, blocking AB	[103]
	intranasal PAR ₂ application in OVA-induced allergy model	inhibited eosinophilia and hyper-responsiveness via COX-dependent generation of PGE ₂	SLIGRL-NH ₂	[26]
PAR ₄ ^{wt}	PAR ₄ antagonism in carrageenan-induced paw inflammation model	reduced edema and granulocyte recruitment	Pepducin	[20]
	PAR ₄ antagonism in mice with systemic inflammation (Shwartzman reaction)	protected against liver and kidney damage, attenuated systemic inflammation	Pepducin	[21]

PAR^{-/-}, PAR-deficient animals; PAR^{wt}, wild-type animals; GN, glomerulonephritis; MPO, myeloperoxidase; TNBS-2,4,6-trinitrobenzene sulfonic acid; blocking AB, blocking antibody; siRNA, small interfering RNA; DSS, dextran sulfate sodium; SP, substance P; COX, cyclooxygenase.

difficile, as mice lacking PAR₂ showed a reduced intestinal, inflammatory response to toxin A [99].

Remarkably, PAR₁ appears to play anti-inflammatory [95] as well as proinflammatory [94] roles in different models of inflammatory bowel diseases. In patients with IBD, PAR₁ is overexpressed in the colon. T and B lymphocytes are involved in the development of PAR₁-induced bowel inflammation [94]. This fact was confirmed by the failure of

PAR₁-AP to induce colonic inflammation in *SCID*^{-/-} and *RAG1*^{-/-} mice [94]. These types of mice are known to lack functional and mature T and B lymphocytes [126]. Moreover, PAR₁ activation exacerbated and prolonged TNBS-induced colonic inflammation, whereas PAR₁ antagonism decreased it [94]. However, an anti-inflammatory role of PAR₁ has been described in Th₂-mediated colitis [95]. It is also interesting to notice that the microspheres with an

encapsulated PAR₁-AP accelerated the healing process of experimental ulcers in rats [127].

These studies clearly demonstrate an important involvement of the adaptive immune system in the PAR-mediated control of colitis. Remarkably, the observed anti-inflammatory or proinflammatory responses stimulated by PAR family members during colonic inflammation appear to be associated with specific effects of these receptors on lymphocyte function or dysfunction, respectively.

The role of PARs in inflammatory processes in the CNS

All four PARs are expressed in the CNS (reviewed in refs. [128, 129]), and their activating proteases could be produced within the brain (trypsinogen IV, tryptase) or infiltrate the brain via the “leaky” blood-brain barrier under inflammatory conditions.

The effects of PAR₁ activation on specific cells in the brain have been summarized recently in a review [130]. However, it is interesting to mention the facts concerning a potential role of PAR₁ activation during CNS inflammation. Encephalitis is a type of brain inflammation caused by viruses or other microbial pathogens. This pathology is also known to be induced by HIV-1. mRNA and protein levels of PAR₁ significantly increase in astrocytes during HIV-induced encephalitis [131]. Moreover, implication of PAR₁-APs in the mouse striatum leads to the activation of astrocytes and microglia cells, indicating an enhanced local inflammatory response [131]. These observations allowed the authors to conclude that activation of astrocyte PAR₁ during HIV-1 infection might contribute to brain inflammation and progress of the infection.

Recent studies performed on animals and also with sections of human brain tissues demonstrated a crucial role of PAR₂ activation during brain inflammation and progression in neurodegenerative diseases. Remarkably, it seems that enhanced PAR₂ expression on neurons of the CNS is neuroprotective in animal models of brain ischemia and inflammatory HIV-associated dementia. On the other hand, increased PAR₂ expression on astrocytes and microglia appears to be associated with neurodegenerative processes in a mouse model of experimentally induced autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS; reviewed in ref. [129]). Striatal implantation of PAR₂-APs significantly inhibited the neurotoxicity induced by the HIV-1-transactivating protein Tat in PAR₂ wild-type mice [101]. Moreover, the severity of neuroinflammation and neuronal damage was higher in PAR₂ knockout animals after Tat implication [101]. On the other hand, enhanced PAR₂ expression on astrocytes and infiltrating macrophages (no changes on neurons were indicated) in EAE and MS mouse models contributes to demyelination and thus, to neurodegenerative processes. In such demyelination, T cells appear to play an important role, and the number of T cells in the spinal cord of PAR₂ wild-type animals was significantly enhanced as compared with knockout mice during EAE [100]. Thus, a role of PAR₂ activation in the process of neuroinflammation in the CNS appears to be dual depending on the disease model and the modification of the expression patterns in the various cells (neuron, astrocyte, glia).

The role of PARs in joint inflammation

A key role of PAR₂ in the development of chronic arthritis was demonstrated recently *in vivo*. In the adjuvant monoarthritis model, joint swelling was inhibited in PAR₂ knockout mice, and the injection of PAR₂ synthetic agonists (2-fuoryl-LIGKV-OH or SLIGRL-NH₂) resulted in joint swelling and hyperemia [88]. It is also important to notice that serine protease-induced joint swelling appears to be mediated via PAR₂ activation, as this response to exogenous application of trypsin and tryptase was absent in PAR₂ knockout mice [103]. Also, the inhibition of PAR₂ up-regulation in synovium (using siRNA technology) reduced joint inflammation in mice [103]. The data indicating a proinflammatory role of PAR₂ during the development of rheumatoid arthritis (RA) in humans were received after the investigation of biopsies of the patients with RA and osteoarthritis (OA). Of note, PAR₂ expression was enhanced in the RA synovium as compared with controls (synovial tissue from patients with OA) [132, 133]. Busso and colleagues [132] studied the effect of PAR₂ deficiency in different types of arthritis animal models, and a significant beneficial effect of PAR₂ gene disruption was found only in an antigen-induced arthritis model. However, the crucial immune cell types mediating the proinflammatory effects triggered by PAR₂ activation during joint inflammation remain unknown.

Yang and colleagues [98] also shed a new light on the role of PAR₁ in a mouse model of antigen [methylated BSA (mBSA)]-induced arthritis. Here, arthritis severity was reduced significantly in PAR₁ null mice as compared with wild-type animals [98]. The notable reduction of total serum anti-mBSA IgG levels in PAR₁ null mice suggested that B cells might be involved in the effects triggered by PAR₁ during joint inflammation. Thus, PAR₁ activation could play an important role in the development of antigen-induced arthritis. Moreover, the effects of the receptor activation might be mediated via modulation of B cell function. The role of PARs in the regulation of B cell function, however, is still poorly understood.

The role of PARs in the progression of skin inflammation and allergy

PAR₁ and PAR₂ are widely expressed in human skin, including keratinocytes, endothelial cells, and sensory nerves [32, 33, 74, 90, 134]. A role of PARs in acute and chronic inflammatory skin diseases such as contact dermatitis (CD) or atopic dermatitis was demonstrated in several works [89, 90, 135].

In a mouse model of experimentally induced CD, PAR₂ plays a crucial role in the development of allergic CD [135]. Indeed, chemically induced ear-swelling responses were significantly suppressed in PAR₂ null mice as compared with wild-type animals. Moreover, the infiltration of immune cells such as neutrophils, T lymphocytes, macrophages, and eosinophils was inhibited significantly in PAR₂-deficient mice [135]. Seeliger and colleagues [89] further explored the role of PAR₂ during cutaneous inflammation. They used a model of experimentally induced allergic as well as toxic CD to demonstrate that responses such as ear swelling, plasma extravasation, and leukocyte adherence were significantly attenuated in PAR₂ null mice as compared with wild-type mice. Remarkably, selectins such as E-selectin seem to be up-regulated after PAR₂ activation in humans. PAR₂ activation was also found to be involved

in CD-induced leukocyte rolling and recruitment into the skin in vivo [89]. PAR₂ has also been shown to mediate itch. In patients with atopic dermatitis, a chronic inflammatory skin disease mediated by keratinocytes, T cells, endothelial cells, as well as mast cells, PAR₂ mediates pruritus and the “triple response of Lewis” (edema, wheal, flare). Thus, serine proteases (tryptase, keratinocyte-derived kallikreins, cathepsins, endothelial-derived trypsin IV) and PAR₂ may be important components in the amplification cycle of inflammation and pruritus in atopic dermatitis [90, 136, 137].

Taken together, the intriguing findings from these studies strongly support the idea that PAR₂ activation plays a proinflammatory role during cutaneous inflammation and pruritus and affects leukocyte recruitment to inflammatory sites within the skin. However, clinical studies using protease inhibitors and PAR₂ antagonists are still necessary to clarify the role of PAR₂ expressed on immune cells and proteases derived from immune cells in the development of atopic dermatitis.

The role of PARs in airway inflammation and allergy

PARs have emerged recently as important effector receptors in the pathophysiology of airway diseases such as asthma, lung edema, and hypersensitivity [70, 138, 139]. There is extensive evidence that PARs are expressed in the airways in a variety of cell types, such as endothelial cells, epithelial cells, smooth muscle cells, neutrophils, and macrophages [70, 140]. The effects of PARs on immune cells in the airways of different species have also been verified.

PAR₁ appears to play a role in the development of lung injury. Intratracheal instillation of bleomycin in a mouse model reflects conditions of acute and chronic fibrotic phases of lung injury. In this model, PAR₁-deficient mice showed a significantly attenuated recruitment of inflammatory cells in the BALF as compared with wild-type mice [141].

A potential role of PAR₂ in lung injury was investigated recently [92]. In this study, PAR₂-AP instilled into airspaces caused a dramatic increase in lung endothelial and epithelial cell permeability to proteins. PAR₂ activation also led to increased leukocyte recruitment in BALF. These findings suggest that PAR₂ activation promotes lung inflammation [92].

Some allergens (house dust mite, cockroach) are demonstrated to activate PAR₂. In some animal models, the importance of the protease activity of particular allergens for their allergic potential has been shown [142, 143]. Ebeling and colleagues [144] reported that PAR₂ activation in the airways at the same time as exposure to inhaled antigens induced allergic sensitization; however, exposure to the same antigen alone induced tolerance. Moreover, this PAR₂-mediated, allergic sensitization in mice appears to be TNF-dependent [144]. A concept that PAR₂ contributes to allergic inflammation of the airways was also supported in the study performed by Schmidlin and colleagues [145]. On the other hand, a protective role of PAR₂ activation was demonstrated recently in an experimentally induced allergic asthma model [146]. Here, the pretreatment with PAR₂-AP significantly inhibited bronchoconstriction as well as airway hyper-responsiveness and modulated the immune response induced by allergic challenge in sensitized rabbits [146]. Additionally, a concept of PAR₂ as a cytoprotective receptor involved in prostanoid-dependent cy-

toprotection in the airways has been supported [147]. Intranasal administration of PAR₂-AP inhibited the development of airway eosinophilia and hyper-responsiveness in allergic mice via a COX-2-dependent generation of PGE₂ [26].

Further investigation of cellular elements of the immune system involved in PAR-associated effects during airway inflammation as well as the identification of novel, natural allergens with proteolytic potential acting via PARs will be helpful for development of novel, anti-allergic therapies.

CONCLUSIONS AND FUTURE PERSPECTIVES

Activation of PARs can be seen to modulate responses of the innate and adaptive immune system under physiological as well as pathological conditions. PARs appear to be involved in leukocyte activation and recruitment toward the site of inflammation or infection. These receptors are also capable of regulating the production of cytokines, chemokines, and ROS by granulocytes, monocytes, or T cells in humans or rodents. Thus, PARs appear to be an integral component of the innate and adaptive immune response and therefore, represent an attractive target for the therapy of inflammatory, infectious, or autoimmune diseases. Notwithstanding, there are still a number of questions to consider in terms of the therapeutic potential of activating or blocking specific PARs, as outlined in the following paragraphs.

For instance, although a significant role for PAR activation in the development of an acute inflammatory response has been established in several animal models, there are still important questions concerning the participation of PARs expressed on immune cells during the progression of chronic inflammation. This issue involves what can be termed “the fourth dimension” of inflammation, wherein the role(s) of PARs at one point in time (e.g., the acute inflammatory response) may differ substantially from the role(s) at later times when the resolution of inflammation (or its lack) results in healing or in a chronic inflammatory process. Indeed, although the activation of PAR₂ can trigger acute inflammation in a number of settings, its activation at times later than the initial insult can be “anti-inflammatory”. Thus, it will be of a key importance to study in-depth the potential roles of PARs 1, 2, and 4 on specific types of immune cells that can be involved at the early and delayed time-points in progression of an inflammatory process. This kind of information will be essential for the development of therapeutic approaches involving the PARs.

The identification of novel endogenous and exogenous (pathogen-derived) proteases, which could signal via PARs under inflammatory conditions in vivo, also represents a prospective research field. Clearly, PAR signaling does not occur in isolation, and the network of signals, conveyed by proteases and inflammatory mediators, must be taken into account for understanding the innate and adaptive response to injury and infection. In this context, it is especially important to extend already accumulated information [93] concerning the transcription factor network of downstream PARs, which could modulate the development of inflammation in vivo. This knowledge will allow the interfering of protease–PAR interactions

during inflammatory diseases at different stages, making possible a wide spectrum of therapeutic approaches.

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