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This review discusses recent progress in understanding how thrombin and protease activated receptors (PAR) influence biological processes, highlighting the detrimental and protective cellular effects of thrombin and its signalling pathways.

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The roles of thrombin and protease activated receptors in inflammation

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Keywords

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Inflammation

Protease Activated Receptor

Introduction

Thrombin, one of key pathway proteins in the coagulation cascade, is a serine protease that initiates the physiological formation of fibrin clots and platelet activation, but has also been implicated in tumour growth, apoptosis, metastasis, pathological tissue remodelling and severe inflammation [1-6]. It is generated by the enzymatic cleavage of two sites on prothrombin by activated Factor X (FXa). Thrombin converts fibrinogen to fibrin, activates other factors in the coagulation pathway (FV, FVIII, FXIII, protein C and thrombin activatable fibrinolysis inhibitor) and can cleave proteins outside the coagulation pathway, such as complement components C3 and C5 (see Fig 1). The ability to elicit responses in a variety of cell types such as leukocytes, platelets and endothelial cells may in part be due to these effects; for instance, many cells have receptors for C3a and C5a [7, 8], although the precise importance *in vivo* of these indirect effects have not been demonstrated. However, thrombin can mediate direct effects on cells through activation of a family of G protein-coupled receptors known as protease-activated receptors (PARs) [9, 10], four of which, PAR-1-4, have been identified to date. Whereas PAR-1, -3 and -4 are activated by proteolytic cleavage of their N-terminal extracellular domains by thrombin [10, 11], PAR-2 is activated by coagulation factors FXa and FVIIa (but not by thrombin) and other proteases such as trypsin, tryptase and proteinase 3 [12-15]. A better understanding of the biochemical pathways involved in thrombin-induced inflammation and of the links between these systems may lead to the identification of new targets for therapeutic proposes. This review focuses on recent advances in our understanding of mechanisms through which thrombin and protease activated receptors influence inflammation and the importance of these in models of diseases.

Role of thrombin in inflammation

The model most widely used to study the role of thrombin and other coagulation proteases in inflammation is that of LPS-induced endotoxaemia, where it is clear from small and large animal studies that inhibiting coagulation inhibits inflammation and prevents significant mortality [16-20].

The role that thrombin plays in this model is complex; fibrin formation and thrombosis has a significant effect on mortality, independently of inflammation [21]. The tools to dissect the fibrin-independent effects of thrombin, in particular the role of PAR signalling, have only relatively recently become available and the insights from some of these studies will be discussed in the next section.

Thrombin has also been shown to be involved in other experimental models of disease including glomerulonephritis [22] and lung fibrosis [23]. In the lung, thrombin mediates expression of mucin, which is sign of airway inflammation and consists of plasma-derived

proteins, mucin glycoproteins and cellular debris, and stimulates expression of tissue factor from nasal epithelial cells through PAR-1 activation. EGF and TNF- α mediated goblet cell activation in the presence of thrombin (leading to secretion of mucin) can be inhibited by anticoagulant activated protein C in rats [24].

Thrombin has been shown to upregulate the expression of cytokines, chemokines and other proteins by different cell types. From human adipocytes, thrombin stimulates secretion of interleukin (IL)-1 β , IL-6, tumour necrosis factor (TNF)- α , monocyte chemoattractant protein (MCP)-1 and vascular endothelial cell growth factor (VEGF) [25]. From urothelial cells, thrombin induces increased release of macrophage migration inhibitory factor (MIF), which can mediate bladder inflammation [26].

There is particular interest in the role that thrombin and other coagulation proteases may play in chronic cardiovascular diseases such as atherosclerosis. On endothelial cells, thrombin can initiate the production of a number of pro-inflammatory mediators including IL-6, IL-8, TGF β , MCP-1, PDGF, ICAM-1 and P-selectin, many through the NF- κ B pathway. This well-described signalling cascade depends upon stimulation of the I κ B kinase (IKK) complex. IKK in turn directs the phosphorylation and subsequent degradation of I κ B. Upon I κ B degradation, NF- κ B is then free to translocate to the nucleus and stimulate transcription of various pro-inflammatory genes. Thrombin induction of the NF- κ B pathway in endothelial cells is known to require prior activation of PKC δ [27, 28]. Using SVEC4-10 mouse endothelial cells transfected with NF- κ B responsive luciferase reporter plasmid, a signalling complex composed of a scaffolding protein (CARMA3), a linker protein (Bcl10), and an effector protein (MALT1), referred to as the CBM signalsome, has been recently identified as a molecular link between PKC and the IKK complex [29]. The CBM signalsome was shown to be an integral part of thrombin dependent NF- κ B pathway in endothelial cells as disruption of this signalsome inhibits the ability of thrombin to induce expression of adhesion molecules ICAM-1 and VCAM-1 and reduces the thrombin-dependent adhesion of monocytes to endothelial cells.

These responses by EC to thrombin can be altered by infection. For instance, following Human cytomegalovirus (HCMV) infection, thrombin induced expression of IL-1 α and macrophage colony stimulating factor (M-CSF) by human umbilical vein endothelial cells was significantly increased, through mechanisms that are incompletely defined, but included increased expression of PAR-3 at the cell surface [30]. This may be relevant as CMV is associated with chronic inflammatory cardiovascular diseases. Thrombin has also been shown to induce expression of IL-6 and CXCL8 from human aortic smooth muscle cells, involving AKT, protein kinase C (PKC), mitogen activated protein kinase and NADPH oxidase signalling [31].

With monocytes or monocyte-derived macrophages, thrombin enhances adhesiveness and increases production of IL-1, TNF- α , IL-6, MCP-1 and IL-10, with downregulation of IL-12 secretion. Oncostatin (OSM) is also produced through a signalling pathway involving

extracellular signal-regulated kinase (ERK1/2) and AP-1 [32]. OSM is a pleiotropic cytokine implicated in the pathophysiology of vascular disease.

At low concentrations, thrombin has anti-inflammatory effects. Incubation of human pulmonary artery endothelial cells (HPAECs) with thrombin at concentrations of 50-75 pM reduced or inhibited LPS-induced permeability changes, neutrophil adhesion and transendothelial migration and expression of cell adhesion molecules such as vascular cell adhesion molecule (VCAM-1), intracellular adhesion molecules (ICAM-1) and E-selectin. Similar effects were reported after pre-treatment of human umbilical vein endothelial cells (HUVECs) with 25-50 pM thrombin [33]. The effects were abrogated by a blocking anti-PAR-1 antibody and were suppressed by LY-294002, a specific cell permeable phosphatidylinositol 3-kinase inhibitor (PI3 kinase), suggesting a role for PAR-1 activation and PI3 kinase pathway in these anti-inflammatory responses in endothelial cells [34, 35].

All this evidence suggests that targeting coagulation proteases might have therapeutic benefit in inflammatory diseases. However, only a single phase III trial, using aPC, showed benefit in human sepsis [36] compared to several others with negative results [37, 38]. One explanation for disappointing results with anticoagulants might be because the doses at which they are can be safely used are insufficient to inhibit PAR-mediated signalling [39]. Additionally, anticoagulants may inhibit thrombin-dependent anti-inflammatory signals. However, newer anticoagulant therapies are now available, including aptamers, direct thrombin inhibitors and PAR-1 antagonists and trials of these agents, to limit inflammation, are eagerly awaited

Protease activated receptors in inflammation

PAR-1

Interactions with S1P receptors in endotoxaemia/sepsis.

Sphingosine 1-phosphate (S1P) is a lysophospholipid that can transmit signals through S1P receptors (S1PR), a family of 5 G-protein-coupled receptors (commonly called S1P₁₋₅) involved in cell differentiation and survival and known to have specific functions in immune cells [40]. S1P signalling pathways have been reported in endothelial cells [41, 42] and human EC express high levels of S1P₁ and P₃. In these cells, S1P₁ is coupled to the G_i/Rac1 signalling pathway, whereas S1P₃ preferentially activates RhoA via G_i, G_{q/11}, and G_{12/13}. The balance between signals through these two pathways plays a role in maintaining vascular integrity and EC barrier function [43].

In the LPS challenge model, early studies suggested significant redundancy between PAR members by showing that deficiency of any single PAR did not confer a survival advantage or protect against inflammation, whereas simultaneous inhibition of multiple PARs did [21].

However, a recent study using PAR-1-deficient mice demonstrated the importance of PAR-1 in this model [44]. In this study, PAR-1^{-/-} mice were protected from the mortality associated with severe endotoxaemia, and wild-type mice treated with either the thrombin inhibitor hirudin or a selective PAR1 antagonist, showed the same protected phenotype. In a similar model of sepsis induced by caecal ligation and puncture (CLP), blocking PAR1 using the PAR1 antagonist reduced lethality and interrupted inflammation in wild-type mice, strongly suggesting that PAR1 alone contributes to lethality after sepsis [44].

In addition, although multiplex cytokine profiles remained unaltered in early LPS-induced inflammatory responses in PAR1^{-/-} mice, they were significantly attenuated 18 h after LPS challenge. Intervention with hirudin or PAR1 antagonist in wild-type mice recapitulated the PAR1^{-/-} phenotype, suggesting that thrombin also amplifies late inflammatory cytokine secretion induced by LPS through specific activation of PAR1.

The role of S1P signalling in LPS-induced inflammation was demonstrated in mice lacking sphingosine kinase 1 (SphK1) (the enzyme that phosphorylates sphingosine), which developed attenuated cytokine profiles similar to those seen in PAR1^{-/-} mice. Stimulation of S1PR in PAR1^{-/-} mice with either an agonist AAL, which activates S1P1 and S1P3, or a selective S1P1 agonist AUY, showed that only the former reversed the attenuated inflammation in PAR1^{-/-} mice, implying that thrombin/PAR-1-dependent amplification after LPS is reliant on S1P3 activation. In support of this, S1P3^{-/-} mice were shown to have attenuated cytokine levels similar to PAR1^{-/-} mice.

Investigating the cellular mechanisms involved, reconstitution of PAR1^{-/-} mice with wild-type bone marrow (BM) restored the late LPS-induced inflammation in PAR1^{-/-} mice, as did adoptive transfer of wild-type BM-derived dendritic cells (DC) in these and in SphK1^{-/-} and S1P3^{-/-} mice. In contrast, transfer of DC from PAR1^{-/-} or S1P3^{-/-} mice into SphK1^{-/-} or S1P3^{-/-} mice had no impact on LPS responsiveness. The authors concluded that PAR1, SphK1 and S1P3 are coupled in an autocrine pathway on DC.

Interestingly, the levels of IL-1 β were increased in mesenteric lymph nodes but conversely reduced in the lungs in PAR1^{-/-}, SphK1^{-/-} and S1P3^{-/-} mice following LPS challenge. IL-1 β levels remained unchanged in the spleen in all knockout strains, consistent with a concept that loss of PAR1-S1P3 signalling specifically contained inflammation in draining lymph nodes. Further work using adoptive transfer of knockout/WT DC into different strains, labelled DCs and selective antagonism of PAR-1 and/or S1PR confirmed that PAR1-S1P3 signalling was necessary for DC to traffic out of draining lymph nodes and that this was responsible for systemic dissemination of endotoxaemia and the lethality associated with endotoxaemia/sepsis.

Thrombin also binds to thrombomodulin following which it is capable of cleaving protein C to generate activated protein C (aPC), a moiety which has pleiotropic effects on coagulation, inflammation, endothelial cells, and immune cells [45-47]. In the context of murine sepsis

or LPS endotoxaemia, aPC acts to reduce mortality, as illustrated by the fact that mice with a thrombomodulin mutation (TM^{Pro}) that prevents effective aPC generation are highly susceptible to LPS-induced death. This susceptibility is reduced when TM^{Pro} is expressed on a PAR-1^{-/-} background, suggesting it is PAR-1 mediated.

In complex with endothelial cell protein C receptor (EPCR) aPC can signal through PAR1. Elegant work using wild-type aPC or mutants with selective anticoagulant (APC149A) or signalling (APC5A) functions have dissected the relative importance of the anticoagulant and signalling functions in inflammation. APC5A protected wild-type but not PAR1^{-/-} or EPCR^{low} mice from dying after LPS, and prevented vascular leakage in a less severe LPS challenge model. Only APC5A but not APC149A rescued TM^{Pro} mice from LPS-induced death, but only in PAR-1⁺ mice. These data indicate that major functions of protein C in this model are due to signalling through PAR-1 and that EPCR/aPC-PAR1 signalling counterbalances the effects of thrombin/PAR1 signalling.

EPCR/aPC-PAR1 signalling *in vitro* is coupled to S1P1 [42]. *In vivo*, a selective agonist for S1P1 receptor protected wild-type, PAR^{-/-}, and TM^{Pro} mice from lethal LPS challenge, and reversed the vascular leakage induced by low dose LPS in wild-type mice administered an inhibitory anti-aPC antibody, or that seen in EPCR^{low} and PAR1^{-/-} mice. These findings suggest that vascular S1P1/S1P3 balance by PAR1 signalling decide the fate of inflammatory diseases [48-50] (see Figure 2).

Importance of PAR-1 activation for leukocyte recruitment.

Besides the amplification role that thrombin/PAR-1 signalling plays in systemic inflammation, our group has been trying to understand whether PAR-1 signalling is required for specific pro-inflammatory functions. Working in a mouse heart-to-rat transplant model, we showed that hearts from monocyte chemoattractant protein-1 (MCP-1) deficient mice survived longer than WT and showed reduced infiltration of NK cells and macrophages under conditions where intravascular thrombosis was inhibited by pre-transplant fibrinogen depletion. This indicated that infiltration of recipient (rat) macrophages and NK cells was significantly impaired in the absence of local (donor heart) chemokines.

Hearts from transgenic mice expressing a tethered hirudin-based fusion protein on endothelial cells showed prolonged survival without NK cell and macrophage infiltration and levels of mouse (donor) MCP-1, -3, and -5 circulating in the rat recipients of these hearts were reduced by >90% compared to rats receiving WT hearts, indicating that intravascular thrombin was required to generate donor chemokines. The same phenomenon was also seen in defibrinogenated recipients of PAR-1^{-/-} hearts or of WT hearts administered a selective PAR-1 antagonist, suggesting that signalling through donor PAR-1 was required to generate the local chemokines for leukocyte recruitment. This was confirmed when a selective PAR-1 agonist, administered to recipients of Tg hearts, increased the levels of circulating mouse chemokines, enhanced macrophage and NK cell recruitment and

shortened survival. After demonstrating similar findings in a model of chemical-induced peritonitis we concluded that thrombin signalling through PAR-1 was necessary to generate local chemokine gradients to initiate leukocyte recruitment [51].

PAR-1 in the nervous system.

PAR1 is expressed throughout the central nervous system on distinct neuronal populations and widely by glial cells in both animals and humans, and has been implicated in the neuronal injury and inflammation in the peripheral nervous system [52-54]. The functional effects of PAR-1 in the nervous system are complex. In some situations, PAR-1 activation is protective. For example, treatment with PAR1 activating peptides such as TRAP6 and TFLLR inhibited the production of pro-inflammatory cytokines TNF- α and IL-6 and promoted the release of anti-inflammatory cytokine IL-10 in mouse microglial cells in response to LPS stimulation. This PAR1-mediated stimulation also enhanced the secretion of 'suppressor of cytokine signalling-3' (SOCS-3), suggesting that PAR1 may be involved in the regulation of inflammatory responses in microglial cells through SOCS induction [55]. However, in other situations, PAR-1 activation is known to be damaging. For instance, deficiency of PAR1 is known to reduce brain infarct volume after arterial occlusion [56]. PAR-1 is also known to potentiate the effects of N-methyl-d-aspartate (NMDA) receptor stimulation, which may partly explain its impact on ischaemic brain injury, where NMDA receptors are known to exacerbate hypoxic injury.

PAR-1 in the gastrointestinal system.

In mouse caecum, both PAR1- and PAR2-activating peptides induced chloride anion (Cl⁻) secretion. PAR1-mediated Cl⁻ secretion can be suppressed by mucosal 5-nitro-2(3-phenylpropylamino) benzoic acid (NPPB), serosal tetrodotoxin (TTX), neurokinin-1 receptor antagonist and removal of Cl⁻ from the bathing solution. PAR2-mediated Cl⁻ secretion can be suppressed by NPPB and removal of Cl⁻, but not by TTX. This suggests that PAR1-mediated secretion may be through activation of secretomotor neurons and activation of the muscarinic and nicotinic receptors [57], while PAR2-mediated secretion may be through activation of receptor on epithelial cells and expression of IL-8 through ERK/RSK90 activation and histone acetylation [57, 58].

C57BL/6 mice fed with a diet deficient in methionine and choline (MCD diet) demonstrated steatohepatitis characterized by macrovesicular hepatic steatosis, pro-inflammatory gene expression and lobular inflammation, which was reduced in PAR1^{-/-} mice, suggesting that PAR1 may contribute to the MCD diet-induced hepatic injury and inflammation[59].

Helicobacter pylori infection, which is associated with gastritis, was reduced in PAR1^{-/-} mice and increased in PAR2^{-/-} mice, and also led to the up-regulation of PAR1 expression and down-regulation of PAR2 expression in wild-type primary gastric epithelial cells. Stimulation of primary gastric epithelial cells from PAR1^{-/-} mice by *H. pylori* resulted in the increased

levels of NF- κ B and the production of pro-inflammatory cytokine macrophage inflammatory protein (MIP)-2, suggesting that PAR1 protects mice from *H. pylori*-induced gastritis possibly through inhibition of MIP-2 production [60].

All these data are interesting, as they implicate PAR-1 in situations where thrombin and coagulation proteases are unlikely to be involved, suggesting alternative activators of PAR-1 might have significant involvement in certain disease states.

Non-thrombin dependent activation of PAR-1

Matrix metalloproteases (MMPs) can cleave the N-terminal domain of PAR1 at a site distinct from the thrombin cleavage site. This process induces p38 mitogen-activated protein kinase (MAPK) signalling and Rho-GTP activation. This thrombin-independent PAR-1 activation is important in platelets, which express several MMPs including MMP-1, -2, -3 and -14, all involved in haemostatic function and platelet survival [61-63]. Exposure of platelets to collagen results in the generation of active MMP-1 from the surface-bound proMMP-1 zymogen, which elicits platelet shape change, calcium mobilization and aggregation through PAR-1. Blockade of MMP1-PAR1 significantly inhibits early thrombogenesis under arterial flow conditions, clot formation and retraction as well as providing protection against collage-dependent platelet activation and thrombosis in animals [64].

MMP cleavage of PAR has also been implicated in the pathogenesis of degenerative brain diseases such as Parkinson's and Alzheimer's, via α -Synuclein, a major protein component of Lewy bodies (the abnormal aggregates found in neurones in these diseases). α -Synuclein has been shown to induce production of NO, reactive oxygen species (ROS) and pro-inflammatory cytokines TNF- α and IL-1 β in primary rat microglia through NF- κ B, AP-1 and MAPK signalling. The effect is inhibited by antagonising PAR-1. MMPs -1, -3, -8 and -9 were also induced, and specific inhibitors of these suppressed the pro-inflammatory effects of α -synuclein. These data suggest that PAR1 activation by MMPs secreted from α -synuclein-stimulated microglia led to the microglial inflammation in an autocrine and/or paracrine manner [65].

PAR-4

A study examining the role of PAR4 in a mouse transient middle cerebral artery occlusion (MCAO) model showed that cerebral infarction volume, blood-brain barrier permeability, and cerebral oedema were significantly reduced in PAR4^{-/-} mice compared with wild-type mice. PAR4^{-/-} mice also showed improved neurologic and motor functions, associated with inhibition of rolling and adhesion of platelets and leukocytes following MCAO, suggesting

that the pathological effects of PAR4 may be related to platelet activation and arterial inflammation [66].

PAR4 is also expressed in the knee joint and in sensory neurons from the joint. PAR4 activating peptide increased joint afferent firing during nonnoxious and noxious rotation of the knee, which was inhibited by a PAR4 antagonist. A bradykinin B₂ receptor antagonist, but not the transient receptor potential vanilloid-1 antagonist, also blocked this increase in firing rate, suggesting that PAR4 mediated sensitization is dependent on B₂ receptor activation [67].

PAR4 has also been implicated in the pathophysiology of inflammatory bowel disease. PAR4 was found to be over expressed in ulcerative colitis (UC) colonic biopsies. Faecal supernatants from UC bowels applied to mouse colons induced colonic paracellular permeability and systemic inflammation and these effects can be blocked by a PAR4 antagonist. They were also blocked by a cathepsin G inhibitor, implicating this as a potential activator of PAR-4 in this model [68].

Finally, PAR4 has been implicated in the in vitro activation of HUVECs by mannose-binding lectin-associated serine protease-1 (MASP-1), a protease of the complement lectin pathway; this elicits a Ca²⁺ response and induces NF-κB nuclear translocation and phosphorylation of p38 MAPK in HUVECs. The effect was dependent on the proteolytic activity of MASP-1, the enzyme was able to cleave a synthetic PAR4 peptide representing the N-terminal sequence of PAR4, and a PAR4 agonist peptide induced a Ca²⁺ response comparable to MASP-1, all of which suggest that this mechanism may be involved in arterial inflammation [69].

PAR-2

PAR-2 has been implicated as a pro-inflammatory receptor involved in several diseases including chronic inflammatory arthritis, dermatitis and colitis. On a cellular level, the pro-inflammatory signalling pathways activated by PAR-2 include phospholipase C, PKC, c-JNK and p38 mitogen-activated protein kinase (MAPK) pathways, resulting in activation of the transcription factors NFκB, peroxisome proliferator-activated receptor-γ and STAT-1 (signal transducer and activator of transcription-1). Very recently, cut homeobox (CUX)-1, a transcription factor present in all metazoans, has been shown to be involved in PAR-2 signalling, specifically in the induction of expression of IL-1α, MMP-10, and cyclo-oxygenase-2 by epithelial cells and fibroblasts. [70].

There is synergy between PAR-2 and toll-like-receptor (TLR)-4 (the receptor that transduces signals after binding of gram-negative LPS)-mediated NFκB signalling, for instance to induce cytokine secretion, on a variety of cell types including macrophages, neutrophils and epithelial cells. Not surprisingly therefore, PAR-2 has also been implicated in the pathology of LPS-induced endotoxaemia, particularly in the renal manifestations, which

characteristically in rodents included glomerular thrombosis and acute renal failure. In this model, a PAR-2 blocking peptide inhibits TNF- α production and reduces fibrin deposition and glomerular thrombosis in rat kidneys, suggesting an important role for PAR2 in LPS-induced renal inflammation and thrombosis [71]. However, markers of renal function such as creatinine and blood urea nitrogen were not altered, suggesting that other, PAR-2 independent mechanisms were operational.

In macrophages, the basis of the synergy between PAR-2 and TLR-4 is a physical association between the two receptors on the cell membrane, as revealed by immunoprecipitation studies [72]. The potential relevance of this was illustrated by the reduced responses of macrophages from TLR-4-deficient mice to a PAR-2 agonist peptide and, the failure of a PAR-2 agonist peptide to induce footpad swelling in TLR-4-deficient mice, compared to WT mice [73], suggesting that TLR-4 activation was necessary for the proinflammatory effects of PAR-2 activation in this model.

Macrophages from PAR-2-deficient mice make an altered response to LPS, with reduced IL-10 secretion but enhanced CXCL1 secretion, skewing the responses to a pro-inflammatory pattern [73]. In WT cells, PAR-2 activation enhanced IL-10, IL-4 and IL-13 secretion and suppressed expression of TNF- α , IL-6, and IL-12, therefore dampening the pro-inflammatory consequences of TLR-4 signalling. In addition, PAR-2 promoted the expression of markers of alternatively activated macrophages (arginase-1 and mannose receptor). Thus, PAR-2 serves as a way to regulate the character of the cellular response to innate activation.

As a further illustration of this concept, activation of PAR-2 by either trypsin or a specific agonist peptide was found to profoundly inhibit the phosphorylation and activity of c-jun N-terminal protein kinase (JNK) induced by TNF α in human skin epithelial cells. This inhibition was PAR2 specific and was reversed by PAR2 antagonist and G_{q/11} inhibitor. The basis was found to be a disruption of TNF-receptor 1 binding to receptor interacting protein and TNF receptor activated death domain, via a protein kinase C dependent mechanism [74]. The relevance of this is that PAR-2 activation itself leads to phosphorylation and activation of JNK, and this has been described as the basis of pro-inflammatory signalling through PAR-2 in numerous cell types. Hence, PAR-2 acts as either pro-inflammatory or anti-inflammatory, depending on the context and character of additional signals received by cells. In this case, since TNF α activation upregulates cell surface expression of PAR-2, this is an example of a negative feedback system to promote cell homeostasis.

Another example of the protective effect of PAR-2 activation was demonstrated by the recent finding of two novel PAR-2 activators, α A-crystallin and α B-crystallin. These are small proteins implicated in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Both have been shown to interact specifically with PAR2, but not PAR-1,-3, or -4. In vitro, activation of PAR2 and increased expression of these proteins protected astrocytes from C2-ceramide- and staurosporine-induced cell death, suggesting that both PAR2 and α -crystallin may be involved in cytoprotection of astrocytes [75]. The authors of this study

suggested that PAR-2 activation by these proteins might be an important mechanism for protecting the brain against damage in chronic degenerative conditions.

Summary and Conclusions

In this review, we have tried to reflect the large volume of recent literature that has addressed the influence of thrombin and the importance of different PAR in inflammation. From this, we believe there are three main things to highlight:

The first relates to the complexity of signalling through PAR-1, exemplified by the different effect of thrombin vs. aPC/EPCR activation, which has been shown to be due to differential S1P1/P3 signalling. It will be extremely interesting to understand how and under what circumstances, these differential effects influence the development or magnitude of harmful or beneficial inflammatory responses. Moreover, it will be important to assess whether this differential signalling can be therapeutically exploited. For example, the development of therapeutic signalling-only aPC analogues is anticipated, with the hope that these will protect the host from inflammation-related vascular leakage and mortality in sepsis. Alternatively, S1P3 antagonists may attenuate DC dependent systemic inflammation and lethality in sepsis.

The second relates to the increasing appreciation of the complexity of the synergistic and inhibitory interactions between PAR-2 and other receptor signalling pathways. For instance, the level of cooperation between PAR-2 and TLR4-mediated signalling pathways indicates that PAR-2 serves as a signalling regulator, involved in fine-tuning of responses to innate activation and in ensuring cellular homeostasis.

The final thing to highlight is the increasing appreciation of thrombin-independent PAR-1 and -4 activation. This area is expected to yield extremely interesting data in the next few years, alongside an increasing appreciation of the importance of proteases for homeostasis and their role in pathology. As the roles of thrombin and related proteases and their associated receptors in inflammation are better defined, it may be possible to develop novel therapeutic agents capable of manipulating specific aspects of the pro- or anti inflammatory pathways involving these molecules.

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Figure 1. Blood coagulation and protein C pathways. Thrombin is produced by the enzymatic cleavage of prothrombin by activated factor X (Xa), which is enhanced by activated factor V (Va). Free thrombin can convert inactive forms of coagulation factors XI, VIII, V to their active forms and fibrinogen to fibrin. In addition, thrombin can promote platelet activation and aggregation by activation of PARs on their surface membrane. Importantly, after binding to the cell membrane protein thrombomodulin, thrombin can activate protein C. Activated protein C (aPC) is an enzyme that inactivates factors Va and VIIIa and signals through PAR-1 after association with endothelial protein C receptor (EPCR). Finally, thrombin can cleave members of the complement system, C3 and C5, though the precise biological relevance of these functions are not known.

Figure 2. PAR1-S1P receptor mediated signalling in the vascular endothelium. Endothelial cells synthesize and display the protein thrombomodulin, which binds thrombin and converts its substrate specificity to cleavage and activation of protein C. Activated protein C binds its endothelial cell protein C receptor, which activates PAR1. PAR1 is coupled to the S1P receptor S1P1. S1P1 signalling is coupled to G_i (the effector is adenylate cyclase, AC) pathway and AKT and RAC 1 activation, which lead to reduce vascular leakage. Thrombin binds to its cell surface receptor PAR1, which promotes RhoGEF association with the S1P receptor S1P3. S1P3 signalling is coupled to G_i , $G_{q/11}$ (the effector, phospholipase C, PLC), and $G_{12/13}$ (the effector, RhoA exchange factor) pathways and activates RhoA. Activated RhoA binds and activates the serine/threonine kinase ROCK, which is involved in the endothelial cell barrier disruption and increases leakage.