Role of Coagulation Factor 2 Receptor during Respiratory Pneumococcal Infections

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Coagulation factor 2 receptor (F2R), also well-known as a protease-activated receptor 1 (PAR1), is the first known thrombin receptor and plays a critical role in transmitting thrombin-mediated activation of intracellular signaling in many types of cells. It has been known that bacterial infections lead to activation of coagulation systems, and recent studies suggest that PAR1 may be critically involved not only in mediating bacteria-induced detrimental coagulation, but also in innate immune and inflammatory responses. Community-acquired pneumonia, which is frequently caused by *Streptococcus pneumoniae* (*S. pneumoniae*), is characterized as an intra-alveolar coagulation and an interstitial neutrophilic inflammation. Recently, the role of PAR1 in regulating pneumococcal infections has been proposed. However, the role of PAR1 in pneumococcal infections has not been clearly understood yet. In this review, recent findings on the role of PAR1 in pneumococcal infections and possible underlying molecular mechanisms by which *S. pneumoniae* regulates PAR1-mediated immune and inflammatory responses will be discussed.

Key Words: *Streptococcus pneumoniae*, Coagulation factor 2 receptor, F2R, Protease-activated receptor 1, PAR1

INTRODUCTION

Community-acquired pneumonia (CAP) is a major cause of death in seniors, and thus economical health care burden due to CAP is rapidly increasing in developed countries, where the portion of senior population is rapidly increasing (1~4). *Streptococcus pneumoniae* (*S. pneumoniae*), which is also commonly called as pneumococcus, is the most common bacterial cause of CAP in seniors (5~7). Severe pneumococcal infections are characterized by severe lung injury at the early stage of infection, which is followed by detrimental intra-alveolar coagulation and neutrophil-profound inflam-

mation (8). Lung injury at the early stage of infection is critical step to initiate intravascular dissemination of pneumococcus and develop detrimental systemic infections, such as bacteremia, meningitis, arthritis, and septicemia (8). We previously reported that type 1 plasminogen activator inhibitor (PAI-1) plays a critical role for protecting against acute lung injury (ALI) and alveolar hemorrhage, and thus loss of PAI-1 results in enhanced mortality due to severe hemorrhagic edema and enhanced dissemination of pneumococcus (8). Later, it has also been found that PAI-1 not only protects against pneumococcal infections by inhibiting tissue injury and alveolar hemorrhage, but also acts as an important regulator of innate immune responses against pneumococcal

infections (9~11). However, underlying molecular mechanisms by which PAI-1 protects lungs and mice against pneumococcal infections have not been clearly understood yet. Moreover, since PAI-1 is the most powerful and effective serine protease inhibitor in coagulation system, and severe pulmonary infections are characterized with pro-coagulative intra-alveolar coagulation, protective effects of PAI-1 against pulmonary bacterial infections have been continuously challenged by clinicians (12, 13).

Coagulation factor 2 receptor (F2R) is a thrombin receptor which belongs to a family of membrane protein receptor protease-activating receptors (PARs) (14). F2R is more commonly called protease-activated receptor 1 (PAR1) and is a key player in coagulation response upon exposure to serine protease thrombin. Recent studies found that PAR1 may contribute to the adverse pathological changes of pneumococcal pneumonia by mediating neutrophil influx into lungs (15~17). In these studies, PAR1 was found to play critical role for regulating immune responses against pneumococcal infections by regulating neutrophil migration into alveolar species (16). Interestingly, although neutrophil influx into alveolar space has been greatly diminished, but anti-pneumococcal effect has not been affected by absence of PAR1 in PAR1 knock-out (KO) mice. These findings suggest the complexity of PAR1-mediated regulation of cellular responses against pneumococcal infections. Taken together with findings from PAI-1 in pneumococcal infections, regulators in coagulation system seems to play diverse roles in regulating host physiological and immune responses against pneumococcus. In this review, we will overview the known roles of PAR1 in regulating bacterial infections, especially respiratory pneumococcal infections, to speculate possible underlying molecular mechanisms and point out the experimental directions to speculate them.

Activation of PAR1 by serine protease thrombin and prokaryotic proteases

PAR1 was initially found as a receptor for platelet thrombin, which is a cell membrane receptor belongs to 7-transmembrane G-protein coupled receptor (GPCR) family protein (14, 18~23). Irreversible cleavage of N-terminal extracellular domain of PAR1 by serine protease thrombin results in forming active N-terminal ligand and auto-activation of PAR1 by binding of active N-terminal ligand to 2nd extracellular loop of PAR1. Since then, several serine protease molecules are reported as activators of PAR1 including plasmin, cathepsin G, granzyme A, and metallomatrix proteases (MMPs) (20~24). Since the first discovery of PAR1, so far four PARs, PAR1, PAR2, PAR3, and PAR4, have been identified, and specific activating proteases and activating molecular mechanisms have been found (20~23). PAR1 is activated by thrombin, plasmin, cathepsin G, granzyme A, leukocyte elastase, gingipain RgpB and HRgpA, and streptokinase-plasminogen complex, and PAR2 is activated by much broad spectrum of mammalian and prokaryotic serine proteases such as trypsin, mast cell tryptase, cathepsin G, protease 3, granzyme A, leukocyte elastase, gingipain-R, gingipain RgpB, thermolysin, serralysin. PAR4 is activated by thrombin, trypsin, cathepsin G, and gingipain RpgB and HRgpA, but activator for PAR3 has mostly unknown yet. Different serine proteases cleave PARs at different but specific site and make unique, specific activating sequence, which called as a tethered ligand (TL) sequence. Specific TL sequences of PARs are SFLLRN (in PAR1), SLIGKY (in PAR2), TFRGAP (in PAR3), and GYPGQV (in PAR4), respectively. Beside these canonical TL sequences, non-canonical proteolytic TL sequences also can be made in PAR1 by non-canonical proteases. MMP1 cleaves PAR1 and makes non-canonical TL sequence PRSFLLRN. Forming NPNDKYEPF by activated protein-C (APC), RNPNDKYEPF by neutrophil elastase (NE), and TLDPRSF by neutrophil proteinase 3 (PR3) also have been found. NE can also cleave PAR2 and make non-canonical TL sequence VLTGKL.

Despite their heterogenic sequences, they all act as auto-activating ligands by binding to their second loop of trans-membrane domain, which results in conformational change in the cytoplasmic C-terminal domain of PARs (25~27). Beside endogenous mammalian cellular proteases, microbial proteases are also reported to activate PARs during infections. Many bacterial pathogens produce several proteases and modulate host immune and inflammatory responses against bacterial pathogens, and some of them are very important

pathogenic factors for inducing diseases (22, 28~31). Among many bacterial proteases, gingipains produced by *Porphyromonas gingivalis* (*P. gingivalis*) are the most well defined PAR activating prokaryotic proteases (28, 30, 31). *P. gingivalis* produces PAR activating gingipains, gingipain R, gingipain RgpB, and gingipain HRgpA. PAR1 is cleaved by gingipains RgpB, gingipain R and gingipain HRgpA cleave PAR2 and PAR4, respectively. Activation of PARs by gingipains is known to contribute to the immune responses activated against *P. gingivalis*. Although specific proteases are not clearly defined yet, many bacterial pathogens exhibit cellular and physiological responses similar to the thrombin-mediated activation of PARs in the absence of thrombin (25, 32~35). These findings suggest that there might be much more prokaryotic proteases directly activating PARs and involved in regulating pathogenicity of their host.

Activation of PARs by their auto-ligands or synthetic agonists, which mimic their specific TL sequence, activates intracellular heterotrimeric G-proteins and transduces distinct intracellular signaling pathways (27, 33, 36). PARs are expressed in multiple cell types including platelet, macrophages, fibroblasts, endothelial cells, and epithelial cells. Therefore, activation of PARs results in many different cellular and biological responses largely depending on the cell types involved in activation and proteases activating PARs (37, 38). Neighboring membrane receptors such as endothelial cell protein C receptor (EPCR), sphingosine 1-phosphate (S1P) receptor, nucleotide-binding oligomerization domain (NOD) receptors, and toll-like receptors (TLRs) are also found to interact with PARs and differentially involved in regulating cellular and biological responses induced by PARs (39~44). Most well-known crosstalk between PARs and cellular membrane receptors is PAR1-EPCR cross-talk. EPCR is a receptor for activated protein C (APC) and EPCR-bound APC activates PAR1 (40, 42, 44). PAR1 activation by EPCR-APC specifically activates Rac1-dependent signaling pathway and inhibits nuclear factor (NF)-κB signaling pathway, thereby inhibiting inflammation and apoptosis. Recent extensive studies uncovered PARs' role in innate immune responses against invading microbes (22, 33). It has been reported that signaling pathways activated by PARs are dependent on the activation of TLRs, and PARs modulate pathogen-associated molecular pattern (PAMP)-inducted activation of TLRs and their downstream signaling pathways, mitogen activated protein kinase (MAPK) and NF-κB signaling pathways. Besides coagulation responses, PARs are thus involved in diverse range of cellular responses including regulation of vascular permeability, immune and epithelial cell apoptosis, production and secretion of pro-inflammatory cytokines and chemokines, and activation and proliferation of fibroblast. By doing these PARs bridge between coagulation and inflammation during infections and determine outcomes from infections.

Indispensable role of PAR1 in respiratory pneumococcal infections

PAR1 is highly expressed in alveolar macrophages, alveolar epithelial cells, alveolar endothelial cells, and also in fibroblast (37, 38). The role of PAR1 in regulating inflammatory responses in lungs remains arguable, where microbial infections were found to activate intra-alveolar coagulation system (45). Since intrapulmonary infections with pneumococcus, which are the most frequent cause of respiratory bacterial infections, are known to upregulate intra-alveolar coagulation, the role of PAR1 in pneumococcal pneumonia has been investigated (17). Upon infection with pneumococcus, PAR1 KO mice showed reduced mortality and diminished pulmonary and systemic pneumococcal outgrowth. Further histopathological studies found significantly diminished inflammatory responses in the lungs of mice with low amount of cytokine expression. In addition, neutrophil migration into lungs was greatly diminished by deficiency of PAR1 in mice. This study proposed possible role of PAR1 in regulating host immune responses against pneumococcus for the first time in animal, and proposed that PAR1 negatively regulates anti-pneumococcal immune responses during pneumococcal pneumonia. Interestingly, despite the significant decrease in the migration of neutrophils into alveoli, which are the most powerful phagocytes against pneumococcus at the early stage of pneumococcal infections in lung, bacterial numbers in lungs and blood are also decreased in PAR1 KO mice.

Further study in other group found that PAR1 expression was upregulated and concomitant increase in coagulation was found upon pneumococcal infection in lung (16). Antagonism of PAR1 with synthetic PAR1 antagonist significantly inhibited inflammatory cell migration into lungs and microvascular permeability. Expressions of cytokine interleukin (IL)-1β and chemokines CCL2 and CCL7 were decreased by PAR1 antagonist, and neutralizations of IL-1β and CCL7, but not CCL2, mitigated neutrophil migration into alveoli upon pneumococcal infections. These findings suggest that PAR1 modulate immune responses against pneumococcal infections by modulating neutrophil migration into alveoli by upregulating IL-1β and CCL7. Interestingly, bacterial growth in lungs and blood has not been affected by PAR1 antagonism and severe reduction in neutrophil migration. Since the authors found depletion of neutrophil resulted in significantly enhanced outgrowth of pneumococcus not only in lungs but also in circulation in the same study, the authors assumed that neutrophils migrated into alveoli under the inhibition of PAR1 activation might be enough to control the pneumococcal growth in lungs. In many cases, inflammation is a double-edged sword, because it is essential part of host defense mechanisms against invading microbes, but detrimental to the host. When negative regulation of inflammation is studied to minimize the adverse inflammatory responses in infections, most difficult question to be answered is if the inhibition of inflammation is beneficial or detrimental to the host. In many *in vivo* studies, inhibition of inflammation seems to be good to protect tissues against tissue injury, but eventually results in uncontrolled outgrowth of microbes. In this context, this unanswered question on this issue should be carefully addressed by further studies. In addition, this study left several questions regarding the molecular mechanisms underlying PAR1 activation by pneumococcus, PAR1-mediated upregulation of cytokine and chemokine expressions, and effects of PAR1 antagonism on pneumococcus-induced mortality.

Proposed acting mechanisms of PAR1 in immune and inflammatory responses against pneumococcal infections

Among many factors in coagulation systems, platelet-activating factor receptor (PAFR) was first a found molecule as an important regulator for the pathogenesis of pneumococcal infections. Phosphorylcholine (PC), which is abundantly expressed in the cell wall of pneumococcus, binds to PAFR in respiratory epithelial cells, and PAFR-mediated attachment of pneumococcus facilitates intraepithelial invasion and transepithelial dissemination of pneumococcus (32, 46, 47). So far no studies are conducted to evaluate the possible crosstalk between PAR1 and PAFR during pneumococcal infections. In tumor biology, it has been discovered that PAR1 upregulates PAFR and PAF expression. It is possible that activation of PAR1 may upregulate expression of PAFR thereby promoting intraepithelial invasion of pneumococcus and facilitate pneumococcal dissemination into circulation. Since deficiency of PAR1 was protective against pneumococcus-induced death in mice and PAR1 antagonism protected mice even under severely decreased intra-alveolar influx of neutrophils, deficiency of PAR1 might have an important role in mitigating dissemination of pneumococcus into circulations, which are the major cause of death in pneumococcal infections.

PAI-1 is one of the most powerful inhibitor of fibrinolysis and major regulator of coagulation. Upon activation of PAR1, platelets produce PAI-1, store it in α-granule, and release it (48). Most of serine protease inhibitors like PAI-1 show substrate specificity, and PAI-1 shows high substrate specificity to serine proteases plasminogen activators (PAs). Although PAI-1 was found to protect lungs and mice against pneumococcal acute lung injury, molecular mechanisms underlying PAI-1-mediated protective effects have not been clearly elucidated (8). None of studies investigated if activation of PAR1 by pneumococcus is induced directly by pneumococcal proteases or indirectly by host-derived endogenous proteases. *S. pneumoniae* expresses serine proteases, such as PrtA and HtrA, which play critical roles on the pathogenesis of pneumococcus (49). Their role in PAR1-mediated regulation of host responses against pneumococcus has to be investigated. In addition, one study reported that streptococcal pyrogenic exotoxin B (SpeB) cleaves PAR1 and inhibits thrombin-induced platelet aggregation thereby promoting dissemination of bacteria (50). This finding suggests that

microbes evolve both positive and negative regulatory mech anisms for controlling PARs signaling pathways. By summarizing, since PAR1 is activated by multiple mammalian serine proteases and also by microbial prokaryotic proteases, it is possible that PAI-1 may exert its protective effect by inhibiting protease-induced PAR1 activation.

For the last, many TLRs are involved in immune and inflammatory responses against pneumococcal infections, including TLR2, TLR4, and TLR9. Since activation of PAR1 modulates downstream signaling pathways of TLRs and PAR1-mediated intracellular signaling pathways are dependent on the activation of TLRs, it is possible that TLRs and PAR1 receptors may cooperate to regulate each other during pneumococcal infections. In addition to TLRs, crosstalk of PAR1 with other PARs, specifically PAR4 might occur during pneumococcal infections (51). Pneumococcal infections in PAR4 KO mice showed higher number of pneumococcus in lungs and also more bacteria disseminated into circulation in PAR4 KO mice (52). Enhanced outgrowth of pneumococcus in PAR4 KO mice was accompanied with higher expressions of cytokines and enhanced pathological changes. These findings suggest that PAR4 play critical role for anti-pneumococcal responses during pneumococcal pneumonia unlike adverse role of PAR1. Since PARs are known to interact and crosstalk each other in many cellular responses, cross-talk between PAR1 with not only TLRs but also with other PARs, PAR2, PAR3, and PAR4, has to be investigated to fully address the role of PAR1 in pneumococcal infections.

CONCLUSIONS

PAR1 is the most effective receptor of thrombin and mediates thrombin-mediated a diverse range of cellular responses. Recent studies found many mammalian and prokaryotic proteases activating PAR1, and variable roles of PAR1 in the pathogenesis of infectious disease have been reported. More recently, PAR1 was found to play critical roles for regulating pathophysiology of pneumococcal infections in lungs, but molecular mechanisms underlying PAR1-mediated regulation of immune and inflammatory responses against pneumococcus have not been studied in

details. Since detailed mechanisms on activating and inhibiting PAR1 have been studied, if we uncover the detailed mechanisms by which PAR1 modulates anti-pneumococcal defense mechanisms, immune and inflammatory responses against pneumococcal infections can be effectively regulated.

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