Editorial

Proteases, Protease-Activated Receptors, and Atherosclerosis

Wolfram Ruf

Coagulation activation by the TF (tissue factor) pathway plays pivotal roles in triggering platelets and precipitating acute coronary syndromes. Although dual antiplatelet therapy is effective in secondary cardiovascular prevention, combining platelet antagonism with low-dose aspirin and the oral coagulation FXa antagonist rivaroxaban has a synergistic clinical benefit over monotherapy in preventing the composite outcome of cardiovascular death, stroke, or myocardial infarction.[1] It is, therefore, of considerable interest to understand the roles of coagulation proteases and their cell signaling effects in the development of atherosclerosis and vascular inflammation. Acute thrombosis in animal models typically requires the combination of FXII contact activation and the extrinsic TF pathway,[2,3] but vascular inflammation and hypertension in the absence of overt intravascular thrombosis can utilize a unique TF-initiated and platelet-assembled thrombin-FXI amplification loop independent of contact pathway FXII.[4] Although atherosclerotic lesions in the mouse are typically not thrombogenic, interference with coagulation nevertheless attenuates lesion development. Genetic manipulation of thrombin generation[5] or deletion of FXI,[6] as well as pharmacological inhibition of FXa[7] or thrombin,[5] reduce atherosclerosis in ApoE (apolipoprotein E) knock-out mice, raising questions about cellular targets and proatherogenic mechanisms of coagulation proteases beyond precipitating intravascular thrombosis.

See accompanying articles on pages 1271 and 1368

Two studies in the current issue of *Arterioscler Thromb Vasc Biol* demonstrate roles for PAR (protease-activated receptor) signaling in atherosclerosis. Rana et al[8] used pharmacological intervention with pepducins, cell-penetrating peptides disrupting intracellular coupling of G protein–coupled receptors, to implicate PAR1 but not PAR2 in lesion development in ApoE$^{-/-}$ mice. Considering the previously demonstrated reduction of plaque burden by coagulation inhibition in this mouse model, it is surprising that thrombin inhibition with the hirudin analogue bivalirudin had no therapeutic benefit. Instead, pharmacological inhibition of MMP (matrix metalloproteinase) 1 attenuated lesion progression with similar efficacy as the PAR1 antagonist. In patients with angiographically confirmed coronary atherosclerotic burden, plasma MMP1 levels were found to be correlated with severity of disease. Macrophage infiltration of atherosclerotic lesions in the mouse was inhibited by the PAR1 pepducin PZ-128 (P1pal-7) and the MMP1 inhibitor FN-439. Although this inhibitor has some activity against MMP13 detected in atherosclerotic lesions, MMP13 plays no apparent role in atherosclerosis development, despite being a relevant PAR1 activator in heart failure.[9] In vitro studies further showed that MMP1–PAR1 signaling is crucial for TNFα (tumor necrosis factor α) induction of VCAM (vascular cell adhesion molecules) and ICAM (intercellular adhesion molecules) in endothelial cells, providing a mechanism for PAR1-mediated support of leukocyte recruitment to the vessel wall (Figure).

Rana et al[8] found no effect of thrombin inhibition, despite documented antithrombotic dosing of bivalirudin, which contrasts with effective lesion reduction by the small-molecule thrombin inhibitor dabigatran given to hyperthrombotic TM^{Pro} (thrombomodulin mutant) mice.[5] Pharmacodynamic differences in the inhibition of free versus cell-bound thrombin and vessel wall penetrance of the low molecular thrombin inhibitor may contribute to the observed differences. In addition, TM^{Pro} mice are impaired in thrombin binding, and consequently, PC (protein C) activation; and activated PC has been implicated in MMP activation.[10] It is, therefore, conceivable that endothelial dysfunction, as mimicked by the TM^{Pro} mouse model, bypasses MMP–PAR1 signaling and enables thrombin-PAR1 driven vascular pathologies. It will be of interest to study and develop new mouse models that are resistant to thrombin cleavage of PAR1 at the canonical Arg^{41}-Ser^{42} bond[11] or to alternative MMP1 cleavage of PAR1 at Asp^{39}-Pro^{40} and MMP13 at Ser^{42}-Phe^{43}, which creates distinct tethered ligands potentially acting as biased agonists.[9]

In a seemingly contradictory second study in this issue, Jones et al[12] demonstrate that PAR2 but not PAR1 deletion attenuates atherosclerotic lesion development in LDLR$^{-/-}$ (low-density lipoprotein receptor) mice. A similar atherosclerosis protection by PAR2 deficiency in ApoE$^{-/-}$ mice[13] essentially excludes different roles for PAR2 in these 2 atherosclerosis models. Knock-out of PAR1 or PAR2 can result in partial compensation for the loss of the reciprocal receptor, as indicated by developmental studies.[14] Such compensation in knock-out mice may obscure receptor participation that is clearly demonstrated by pharmacological approaches. PAR receptor cross talk is indeed likely, because PAR1 and PAR2 form functional heterodimers in endothelial cells,[15] and the antiatherogenic PAR1 pepducin PZ-128 (P1pal-7) is known to inhibit PAR1/PAR2 heterodimer signaling.[16] In addition, an agonistic PAR1 pepducin produced injury-induced smooth

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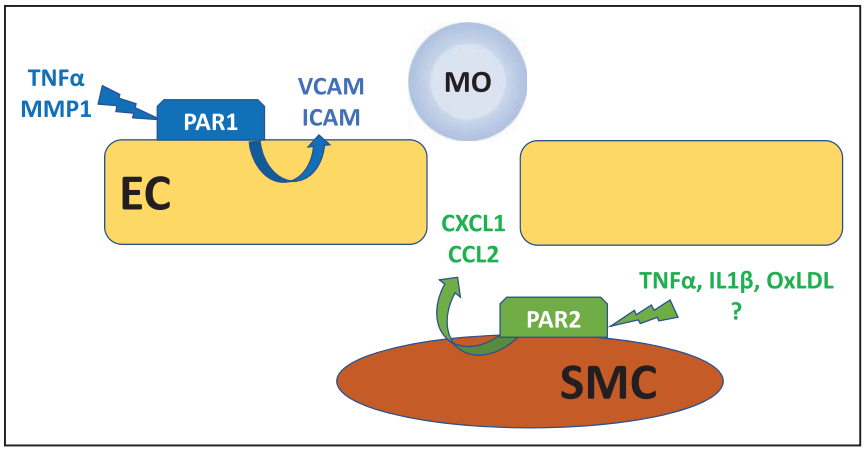

Figure. Proposed contributions of endothelial cell (EC) MMP (matrix metalloproteinase) 1–PAR (protease-activated receptor) 1 and smooth muscle cell (SMC) PAR2 signaling in monocyte (Mo) recruitment in atherosclerosis. CCL2 indicates C-C motif chemokine ligand 2; CXCL1, C-X-C motif chemokine ligand 1; ICAM, intercellular adhesion molecule; IL-1β, interleukin 1β; OxLDL, oxidized low-density lipoprotein; TNFα, tumor necrosis factor α; and VCAM, vascular cell adhesion molecule.

muscle cell hyperplasia dependent on PAR1 and PAR2 expression,[17] demonstrating that pepducins can modulate vessel wall PAR1/PAR2 heterodimer signaling.

The study by Jones et al[12] adds to the increasing evidence that PAR2 is central to inflammatory processes, participating in innate immune signaling by toll-like receptor 4[18,19] and promoting metabolic inflammation in obesity.[20] Although stromal and hematopoietic cell TF-PAR2 signaling contributes to weight gain and insulin resistance in obesity, atherosclerosis development is primarily driven by the radiation-insensitive vascular compartment. PAR2-deficient vascular smooth muscle cells stimulated with PAR2 agonist, TNFα, or IL-1β (interleukin 1β) express lower levels of chemokines CCL2 (C-C motif chemokine ligand 2) and CXCL1 (C-X-C motif chemokine ligand 1), and consequently, monocyte migration in vitro and vessel wall infiltration is reduced.[12] Future studies with cell type–specific deletion of PAR2 are necessary to confirm the role for smooth muscle cell PAR2 in regulating leukocyte recruitment and identify potential roles of endothelial cell PAR2 in the upregulation of endothelial cell adhesion molecules by MMP1–PAR1 signaling proposed by Rana et al.[8]

These studies not only provide important new insights into the contributions of protease-signaling pathway to the development of atherosclerosis but also demonstrate the need to evaluate these complex processes by combinations of pharmacological and genetic approaches. Taken together, these studies show that the key vascular signaling transducers for coagulation proteases, PAR1 and PAR2, both participate in the development of atherosclerosis and can be targeted for the attenuation of lesion progression. It is also clear that activation of PARs in the vascular compartment may involve interconnected proteolytic cascades and is not restricted to coagulation proteases. Understanding the limitations of target selective clinical anticoagulants, that is, thrombin and FXa inhibitors, and the contributions of these proteases to PAR activation in vascular and immune cells will be essential for predicting long-term benefit of antithrombotic therapy in secondary cardiovascular prevention.

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Disclosures

None.

References

1. Eikelboom JW, Connolly SJ, Bosch J, et al; COMPASS Investigators. Rivaroxaban with or without aspirin in stable cardiovascular disease. *N Engl J Med*. 2017;377:1319–1330. doi: 10.1056/NEJMoa1709118.
2. von Brühl ML, Stark K, Steinhart A, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med*. 2012;209:819–835. doi: 10.1084/jem.20112322.
3. Furlan-Freguia C, Marchese P, Gruber A, Ruggeri ZM, Ruf W. P2X7 receptor signaling contributes to tissue factor-dependent thrombosis in mice. *J Clin Invest*. 2011;121:2932–2944. doi: 10.1172/JCI46129.
4. Kossmann S, Lagrange J, Jackel S, et al. Platelet-localized fxi promotes a vascular coagulation-inflammatory circuit in arterial hypertension. *Sci Transl Med*. 2017;9:eaah4923.
5. Borissoff JI, Otten JJ, Heeneman S, et al. Genetic and pharmacological modifications of thrombin formation in apolipoprotein e-deficient mice determine atherosclerosis severity and atherothrombosis onset in a neutrophil-dependent manner. *PLoS One*. 2013;8:e55784. doi: 10.1371/journal.pone.0055784.
6. Shnerb Ganor R, Harats D, Schiby G, Gailani D, Levkovitz H, Avivi C, Tamarin I, Shaish A, Salomon O. Factor XI deficiency protects against atherogenesis in apolipoprotein E/factor XI double knockout mice. *Arterioscler Thromb Vasc Biol*. 2016;36:475–481. doi: 10.1161/ATVBAHA.115.306954.
7. Hara T, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Nishimoto S, Yagi S, Yamada H, Soeki T, Wakatsuki T, Shimabukuro M, Sata M. Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. *Atherosclerosis*. 2015;242:639–646. doi: 10.1016/j.atherosclerosis.2015.03.023.
8. Rana R, Huang T, Koukos G, Fletcher EK, Turner SE, Shearer A, Gurbel PA, Rade JJ, Kimmelstiel CD, Bliden KP, Covic L, Kuliopulos A. Noncanonical matrix metalloprotease 1–protease-activated receptor 1 signaling drives progression of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2018;38:1368–1380. doi: 10.1161/ATVBAHA.118.310967.
9. Austin KM, Covic L, Kuliopulos A. Matrix metalloproteases and PAR1 activation. *Blood*. 2013;121:431–439. doi: 10.1182/blood-2012-09-355958.
10. Jackson MT, Smith MM, Smith SM, Jackson CJ, Xue M, Little CB. Activation of cartilage matrix metalloproteinases by activated protein C. *Arthritis Rheum*. 2009;60:780–791. doi: 10.1002/art.24303.
11. Sinha RK, Wang Y, Zhao Z, Xu X, Burnier L, Gupta N, Fernández JA, Martin G, Kupriyanov S, Mosnier LO, Zlokovic BV, Griffin JH. PAR1 biased signaling is required for activated protein C in vivo benefits in sepsis and stroke. *Blood*. 2018;131:1163–1171. doi: 10.1182/blood-2017-10-810895.
12. Jones SM, Mann A, Conrad K, Saum K, Hall DE, McKinney LM, Robbins N, Thompson J, Peairs AD, Camerer E, Rayner KJ, Tranter M, Mackman N, Owens AP III. PAR2 (protease-activated receptor 2) deficiency attenuates atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2018;38:1271–1282. doi: 10.1161/ATVBAHA.117.310082.
13. Zuo P, Zuo Z, Zheng Y, Wang X, Zhou Q, Chen L, Ma G. Protease-activated receptor-2 deficiency attenuates atherosclerotic lesion progression

and instability in apolipoprotein E-deficient mice. *Front Pharmacol*. 2017;8:647. doi: 10.3389/fphar.2017.00647.
14. Camerer E, Barker A, Duong DN, et al. Local protease signaling contributes to neural tube closure in the mouse embryo. *Dev Cell*. 2010;18:25–38. doi: 10.1016/j.devcel.2009.11.014.
15. Trejo J, Coughlin SR. The cytoplasmic tails of protease-activated receptor-1 and substance P receptor specify sorting to lysosomes versus recycling. *J Biol Chem*. 1999;274:2216–2224.
16. Yoon H, Radulovic M, Wu J, Blaber SI, Blaber M, Fehlings MG, Scarisbrick IA. Kallikrein 6 signals through PAR1 and PAR2 to promote neuron injury and exacerbate glutamate neurotoxicity. *J Neurochem*. 2013;127:283–298. doi: 10.1111/jnc.12293.
17. Sevigny LM, Austin KM, Zhang P, Kasuda S, Koukos G, Sharifi S, Covic L, Kuliopulos A. Protease-activated receptor-2 modulates protease-activated receptor-1-driven neointimal hyperplasia. *Arterioscler Thromb Vasc Biol*. 2011;31:e100–e106. doi: 10.1161/ATVBAHA.111.238261.
18. Nhu QM, Shirey K, Teijaro JR, Farber DL, Netzel-Arnett S, Antalis TM, Fasano A, Vogel SN. Novel signaling interactions between proteinase-activated receptor 2 and toll-like receptors in vitro and in vivo. *Mucosal Immunol*. 2010;3:29–39. doi: 10.1038/mi.2009.120.
19. Liang HP, Kerschen EJ, Hernandez I, Basu S, Zogg M, Botros F, Jia S, Hessner MJ, Griffin JH, Ruf W, Weiler H. EPCR-dependent PAR2 activation by the blood coagulation initiation complex regulates LPS-triggered interferon responses in mice. *Blood*. 2015;125:2845–2854. doi: 10.1182/blood-2014-11-610717.
20. Badeanlou L, Furlan-Freguia C, Yang G, Ruf W, Samad F. Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation. *Nat Med*. 2011;17:1490–1497. doi: 10.1038/nm.2461.

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