A *Pad* 4 Plaque Erosion

Oliver Soehnlein, Venetia Bazioti, Marit Westerterp

Despite the development of new intervention strategies, acute coronary syndromes (ACS) are among the leading cause of global mortality. ACS is predominantly caused by plaque rupture, which is the consequence of inflammatory processes within the atherosclerotic plaque that contribute to expansion of the necrotic core and thinning of the fibrous cap. However, with current therapeutic strategies targeting traditional risk factors such as plasma LDL-C (low-density lipoprotein cholesterol) levels and hypertension, the relative contribution of plaque erosion to ACS is on the rise. Plaque erosion is characterized by endothelial cell desquamation and small intraluminal thrombi, and now accounts for ≈25% to 30% of all ACS.[1]

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Neutrophils are the most abundant white blood cells and form the first line of defense against microbes and bacteria. In addition to their key role in host defense, studies over the past decade have revealed that neutrophils are of major importance to cardiovascular pathologies, including atherosclerosis, neointima formation, and myocardial infarction.[2–4] Neutrophils mainly act through their granule proteins which can be released directly or when bound to neutrophil extracellular traps (NETs), a network consisting of neutrophil-derived DNA, and proteins of nuclear, granular, and cytosolic origin. Markers of NETs have been associated with coronary stenosis and plaque erosion in humans[5,6] and NET formation (NETosis) has been suggested to promote atherogenesis in mice.[7,8] In addition, recent studies have shown that NETs play a key role in regulating processes occurring at the luminal side of blood vessels, including entrapment of metastatic tumor cells,[9] vaso-occlusion,[10] and endothelial permeability.[2]

In this issue of *Circulation Research*, Franck et al[11] report on the role of PAD4 (peptidylarginine deiminase 4), an enzyme important during histone citrullination and chromatin decondensation, in atherosclerosis and plaque erosion. *Ldlr*$^{-/-}$ mice were transplanted with *Pad4*$^{-/-}$ or wild-type bone marrow and fed a high-cholesterol (1.25%) diet for a period of 5 or 10 weeks. While hematopoietic *Pad4* deficiency clearly decreased histone citrullination in atherosclerotic lesions, lesion area and composition were not affected at both time points.[11] Subsequently, the authors used their previously developed acute carotid artery injury model[12] to evaluate the role of PAD4 in plaque erosion in mice. Similar to findings in human plaques,[6] NETs were in close contact with luminal endothelial cells during plaque erosion in this model, which was associated with endothelial DNA fragmentation.[11] Strikingly, hematopoietic *Pad4* deficiency preserved endothelial permeability and continuity while suppressing endothelial cell death, suggesting protection from plaque erosion. Fibrinogen and tissue factor staining were also decreased, reflecting smaller intraluminal thrombi.[11] This study is the first to demonstrate that PAD4 and histone citrullination promote endothelial erosion, with potential consequences for atherothrombosis. These observations could have important implications for a subset of ACS in humans and may suggest PAD4 as a therapeutic target.

While hematopoietic *Pad4* deficiency did not affect blood leukocyte numbers or neutrophil chemotaxis to endothelial cells in an in vitro assay, it decreased neutrophil adherence to the endothelium during endothelial erosion. NETosis and neutrophil-endothelium adherence correlated in the acute injury model.[11] In this model, endothelial expression of TLR2 (Toll-like receptor 2) is enhanced because of disturbances in blood flow. TLR2 promotes endothelial erosion by promoting neutrophil adherence.[12] The present study implies that PAD4, downstream of neutrophil adherence promotes endothelial erosion (Figure).[11] TLR2 expression on endothelial cells also colocalizes with NETs in erosion-prone atherosclerotic human plaques.[6] Together, these data[6,11] may suggest a role for NETs in plaque erosion in humans. The complement pathway, which has been associated with endothelial damage previously, was suggested to mediate NET-induced endothelial erosion[11]; however, this would warrant further investigation.

The question remains as to how NETosis is activated during endothelial erosion. NETosis has been studied extensively in vitro; however, these assays use PMA (phorbol 12-myristate 13-acetate), IL (interleukin)-8, lipopolysaccharide, or calcium ionophores, and the relevance of these compounds for NETosis at the luminal side of atherosclerotic lesions may be questionable. Activated platelets have previously been reported to induce NET release through direct P-selectin–mediated interaction with neutrophils, or via soluble factors, including HMGB1 (high mobility group B1) and CCL5/CXCL4 (chemokine (C-C motif) ligand 5/CXC chemokine ligand 4) chemokine heteromers.[2] Because platelets have emerged as important players during various stages of atherosclerosis, their contribution to luminal NET release is likely. In addition, the ligation of neutrophil integrins with endothelial cell adhesion molecules may also prompt NET release.[2] Hence, it becomes important to understand to what extent the endothelium generated in the

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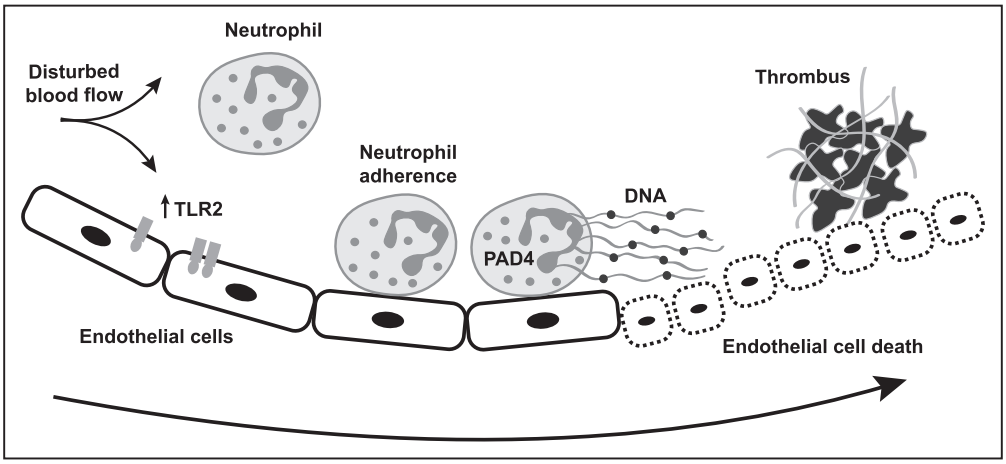

Figure. Model of Pad4 (peptidylarginine deiminase 4) promoting endothelial erosion. At sites of disturbed blood flow, TLR2 (Toll-like Receptor 2) expression is upregulated on endothelial cells, promoting neutrophil adherence. Pad4 is involved in the release of neutrophil extracellular traps (NETs) that stimulate endothelial cell death and thrombus formation.

model used in this study mimics human arterial endothelium prone to desquamation. Sensors expressed by neutrophil detecting low shear stress may also enhance NET release at the luminal side of large arteries. Finally, high plasma LDL-C levels could induce NET release. Further studies will be required to identify triggers for NET release in vivo in the context of plaque erosion.

Recent studies have shown that activation of the NLRP3 (NLR family, pyrin domain containing 3) inflammasome in myeloid cells, which enhances secretion of proatherogenic IL-1β and IL-18, promotes neutrophil accumulation and NETosis in atherosclerotic plaques of *Ldlr*$^{-/-}$ with myeloid deficiency of the cholesterol transporters ABCA1 and ABCG1 (ATP binding cassette A1 and G1).[13] ABCA1 and ABCG1 mediate cholesterol efflux to high-density lipoproteins. Deficiency of *Abca1/Abcg1* enhances cholesterol accumulation in myeloid cells, including neutrophils, which activates the NLRP3 inflammasome. In vitro studies in neutrophils suggested that deficiency of *Abca1/Abcg1* did not affect NETosis via cell-intrinsic effects; indicating that NETosis in atherosclerotic plaques was rather the consequence of inflammasome activation in monocytes and macrophages.[13] Indeed, IL-1β, a product of inflammasome activation, promotes NETosis in a gout model.[14] These observations suggest that not only secretion of inflammatory mediators by platelets, but also by myeloid cells, may promote NETosis, although precise molecular mechanisms warrant further studies.

In yet another study, it was recently found that IL-1β may not just trigger NET release, but that NETs may, in fact, contribute to the production of lesional IL-1β. NETs are to a large degree composed of dsDNA which can be sensed by the AIM2 (absent in melanoma 2) inflammasome. Both dsDNA and AIM2 were shown to accumulate in advanced stages of atherosclerotic plaques. *AIM2* deficiency or its therapeutic inhibition reduced lesional IL-1β by ≈50% and IL-18 by 60%. As a consequence, atherosclerotic lesions appeared more stable, characterized by thicker fibrous caps and smaller necrotic cores.[15]

In conclusion, the study by Franck et al[11] identifies an important role for PAD4 in plaque erosion, which may have broad clinical implications for ACS. Challenges for the future would entail the identification of pathways (possibly arterial-specific) promoting NETosis as targets for therapy.

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Disclosures

None.

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