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EDITORIAL COMMENT

Implications of Targeting Neutrophil Extracellular Traps in Aortic Aneurysms*

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Abdominal aortic aneurysm (AAA) is a chronic vascular inflammatory disease that manifests as a local enlargement of the abdominal aorta, typically in the infrarenal aorta.[1] Currently, surgical or endovascular intervention is the only viable option to prevent rupture, and effective pharmacologic treatments for AAA are urgently required for this patient cohort. The molecular pathology of AAA is complex and multifactorial, involving the interplay of signaling events between immune cells and parenchymal cells leading to chronic inflammation and vascular remodeling. An inflammatory cell infiltrate, smooth muscle cell proliferation and plasticity, degradation of the elastic media of the atheromatous aorta, and the production and activation of various proteases and cytokines contribute to the development of this disorder. Recent studies have focused on the pathologic changes involving marked infiltration of inflammatory cells, such as neutrophils, leading to formation of neutrophil extracellular traps (NETs) that contribute to the inflammatory milieu of the aortic tissue microenvironment. The formation of NETs generally represents a host defense mechanism for mitigating pathogenic responses via the extracellular network comprising bactericidal proteins. Although NETosis is beneficial for the entrapment and clearance of pathogens, the induction of NETosis in sterile inflammation has also been reported and signifies a contributory mechanism to the pathogenesis of AAA.[2]

Activated neutrophils can lead to dysfunction between the aortic endothelial layers and neutrophils that contributes to vascular inflammation. As AAA progression is characterized by infiltrating leukocytes, resulting changes in parenchymal cells, such as endothelial cells and smooth muscle cells, lead to the hallmarks of this chronic vascular pathology. Characteristic features include fragmentation of the elastic fibers and a decreased concentration of elastin during aneurysmal growth until the time of aortic rupture. Subsequently, elastic and collagen fibers are degraded by proteolytic enzymes by matrix metalloproteinases (MMPs) that can be secreted by activated smooth muscle cells and/or lymphomonocytic infiltrating immune cells as well as secondary to plasminogen activators. Leukocyte recruitment into the aortic wall is promoted by elastin degradation fragments as well as proinflammatory cytokines and chemokines, derived from parenchymal as well as inflammatory cells such as M1 macrophages and $CD4^+$ T cells. An additional and prominent hallmark of this disease process that has gained more attention in recent molecular studies is the presence of mural thrombus in most patients with AAA. The role of thrombi in initiating or contributing to the ongoing process of inflammation and cell death, especially ferroptosis (excessive iron-mediated cell death), is currently a topic of significant interest in this field. As blood flow is maintained in aortic aneurysms, compared with other arterial occlusive diseases, it contributes to thrombus components, causing persistent remodeling activity of the vascular tissue. The presence of plasminogen and its activator in the vascular thrombus of the aneurysm wall likely contributes to local generation of plasmin, which can increase MMP activity, which is known to considerably alter smooth muscle cell proliferation and result

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in aortic remodeling. Importantly, recent evidence suggests neutrophil trapping and MMP activity within the aneurysmal thrombus, which underscores a correlation between these processes during aortic inflammation and vascular remodeling of the aneurysm.[3] However, the role of antiplatelet or antithrombotic therapy for patients with AAA is controversial because of increased risk for mortality, endoleak, and/or reintervention and merits further investigation. Therefore, it becomes imperative to investigate the contribution of other components, such as NETs, that could be associated with thrombus formation and AAA progression.

NETs are DNA structures released because of chromatin decondensation and spreading, which can significantly influence the surrounding tissue microenvironment. The genesis of NETs classically commences with the activation of neutrophils through the recognition of stimuli, and activation of reactive oxygen species-dependent pathway involving the nicotinamide adenine dinucleotide phosphate oxidase (NOX) complex through protein kinase C/Raf/MERK/ERK. The major characteristics of NETs are adherent proteins, including histones and components with bactericidal activity such as elastase, myeloperoxidase (MPO), cathepsin G, gelatinase, proteinase 3, and peptidoglycan-binding proteins, to mitigate pathogenic activity.[4] The release of NETs begins with the activation of peptidyl arginine deiminase 4 (PAD4), which causes histone citrullination, extensive chromatin decondensation, and nuclear localization of granular enzymes (eg, MPO, neutrophil elastase). Recent studies have shown the efficacy of PAD4 inhibitors to reduce murine AAA progression and aortic rupture, underscoring the relevance of NETosis in the pathogenesis of AAA.[2] Moreover, studies in patients with AAA have demonstrated that plasma concentrations of NETosis parameters such as MPO, citH3, and cell-free DNA levels were significantly increased compared with healthy donors. These studies raise the interesting prospect of using NET-specific biomarkers as a predictor of disease progression and highlight the prospective relevance of developing therapeutics targeted toward mitigating NET genesis or progression in vascular diseases.

In a study reported in this issue of *JACC: Basic to Translational Science*, Ibrahim et al[5] investigated the efficacy of mitigating AAA progression via targeted pathways and molecules that are known to be involved in NET formation. Two distinct mechanistic aspects of blocking NETs were investigated: mitigating upstream signaling events to prevent NET induction via a Nox2-dependent pathway or a PAD4-dependent pathway and inactivating molecular components of preformed NETs via deoxyribonuclease I (DNase I) or extracellular histones. The investigators used 2 established murine models of AAA: an angiotensin II (Ang II) model that frequently involves an intramural thrombus resembling the sequelae of human established disease and a periadventitial elastase model that is reflective of initial disease formation and expansion without thrombus formation. Notably, NETs accumulated in the intramural thrombus in the Ang II model, and anti-NET treatment was more potent in decreasing aneurysm growth in the Ang II model than the elastase model. Furthermore, upstream NET inhibition using PAD4 or Nox2 inhibitors was observed to be more effective than downstream inhibition by DNase I, attenuating aneurysmal growth in the Ang II model, particularly in mice that developed dissection and intramural thrombus. These results are consistent with those of previously reported studies showing that inhibition of PAD4, but not DNase I, can attenuate experimental murine AAAs.[2] However, this study used another compound, GSK484, to target histone citrullination as an upstream inhibitor of NETs and displayed a preserved contractile smooth muscle cell phenotype and inhibition of AAA growth. These interesting observations implicate the correlation of accumulation of NETs and thrombosis, which may contribute to the progression and pathogenesis of the aneurysmal aorta and impending rupture. Furthermore, inhibiting the NET induction process was found to be more effective than neutralizing already formed NET products. This underlines the importance of early detection and mitigation of NET formation in AAAs, which highlights the importance of this pathway in vascular pathologies.

Although this study highlights the importance of targeting early NET formation, clinical translation can be challenging because of the fact that pre-emptive treatment strategies for a chronic vascular disease in an elderly population can be difficult compared with therapeutic options for the attenuation of a preformed aneurysmal growth. Moreover, this study did not delineate the sequential mechanism of proposed events involving thrombus formation and NET deposition to determine if NETs promote the thrombus formation or if the formation of thrombus allows NET-mediated vascular inflammation in the pathophysiology of AAAs. This is important to decipher because if thrombi propagate NET formation, then the inhibition of NETs may not lead to sustained resolution of vascular injury, especially in a chronic

vascular pathology. Accordingly, it is plausible that platelets may play a pivotal role in thrombus formation, which allows prolonged NET deposition and vascular toxicity, and it is crucial to consider that damage-associated molecular pattern molecules such as high-mobility group box 1 protein-secreting platelets can also be potent inducers of NET formation in not only infectious pathologies but also sterile inflammation. Therefore, the sequential role of platelets and thrombotic events in the genesis and/or progression of NETs in the context of chronic vascular pathologies such as AAA should be considered in further investigations. Furthermore, detailed mechanistic investigations are required to decipher the effect of compounds that inhibit PAD4, such as GSK484, on neutrophil-platelet aggregates, thrombosis formation, effect on NOX2-mediated reactive oxygen species, and regulation of immune-parenchymal cell crosstalk during chronic AAA formation and aortic rupture.

In summary, these recent findings suggest a "NET impact" on the progression of vascular pathologies, thereby suggesting a much required pharmaceutical approach for management of diseases such as aortic aneurysm. Thus, targeting multifaceted components of antithrombotic and NET-specific approaches may be more efficacious to prevent AAA progression and aortic rupture and significantly improve clinical outcomes.

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REFERENCES

1. Sakalihasan N, Limet R, Defawe OD. Abdominal aortic aneurysm. *Lancet*. 2005;365:1577–1589.

2. Fernandez-Ruiz I. Inflammation: NETs are involved in AAA. *Nat Rev Cardiol*. 2018;15:257.

3. Fontaine V, Jacob MP, Houard X, et al. Involvement of the mural thrombus as a site of protease release and activation in human aortic aneurysms. *Am J Pathol*. 2002;161:1701–1710.

4. Delgado-Rizo V, Martinez-Guzman MA, Iniguez-Gutierrez L, Garcia-Orozco A, Alvarado-Navarro A, Fafutis-Morris M. Neutrophil extracellular traps and its implications in inflammation: an overview. *Front Immunol*. 2017;8:81.

5. Ibrahim N, Bleichert S, Klopf J, et al. Reducing abdominal aortic aneurysm progression by blocking neutrophil extracellular traps depends on thrombus formation. *J Am Coll Cardiol Basic Trans Science*. 2024;9(3):342–360.

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