Thrombi and Neutrophils

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More than a century ago, in 1881, Bizzozero observed platelets and their role in primary hemostasis. Since this founding study, it has been shown that, in addition to the scaffold of platelets and fibrin, other proteins and cells play an important role in thrombus formation and stabilization. One can already observe in the previous drawings of Bizzozero that leukocytes and red blood cells are trapped within the thrombus. We know now that thrombi are actually heterogeneous and can take on multiple forms both in the arterial and venous systems. As a consequence of this diversity, thrombi can have a variable effect on the development and outcome of cardiovascular diseases. In fact, whereas occlusive thrombi can abruptly cause dramatic acute ischemic events, nonobstructive thrombi, like those found in abdominal aortic aneurysms, can slowly and progressively degrade the vessel wall via convection of blood-borne and leukocyte-derived proteases. Furthermore, thrombus heterogeneity bears important clinical implications because the efficacy of thrombolysis in the treatment of acute ischemic events is highly dependent on thrombus composition and structure. For example, both platelet-rich thrombi and aged thrombi are known to be more resistant to the gold-standard thrombolytic agent, recombinant tissue plasminogen activator (r-tPA). Because of these barriers to r-tPA–induced thrombolysis and given that r-tPA targets the fibrin scaffold, it has been proposed that targeting other thrombus components (eg, Von Willebrand factor) could help to improve the efficacy of thrombolysis.

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In recent years, neutrophil extracellular traps (NETs) have been identified as major triggers and structural factors of various forms of thrombosis, including infection-induced thrombosis, deep vein thrombosis, and cancer-associated thrombosis. NETs are extracellular webs primarily composed of DNA from neutrophils that ensnare pathogens but also cause platelet activation and aggregation. It should be noted that the discovery of NETs and of their role in thrombosis somewhat rehabilitates the theories of Schmidt and Mantegazza, who both attributed triggering and structural roles of leukocytes and leukocyte destruction in thrombus formation before being eclipsed by Bizzozero and his discovery of platelets a few years later. Although most of the work on NETs has been focused on the venous compartment, NETs have also been identified in the arterial compartment, such as in the thrombus of human aortic aneurysms. Therefore, the contribution of NETs to thrombus formation and to thrombotic events is likely far from being restricted to venous thrombosis, and NETs could, in consequence, be promising targets for improved thrombolysis under various thrombotic conditions. The results of Mangold et al13 presented in this issue of Circulation Research strongly support this hypothesis. In this translational clinical investigation, the authors explored the relationship between the presence of neutrophils and NETs in human obstructive coronary thrombi and myocardial infarction evolution. Precisely, this study shows that the NET burden in culprit coronary thrombi is positively correlated with the infarct size and negatively correlated with ST-segment resolution. They further show that, on the contrary, DNase activity correlates negatively with coronary thrombus NET burden and with infarct size. This study therefore points to an aggravating role of neutrophils and NETs in ST-elevation acute coronary syndrome, an effect that could be because of the increased resistance of the NET-loaded thrombus to fibrinolysis. The authors indeed show that DNase accelerates the lysis of coronary thrombi by tPA in vitro. Interestingly, a detrimental action of NETs was recently shown in a mouse model of myocardial ischemia/reperfusion. Together, this study and that by Mangold et al13 thus suggest that targeting NETs with DNase could be beneficial both to increase the efficacy of thrombolysis and to reduce the ischemia-related cardiac damage. NETs are indeed coated with neutrophil proteolytic and oxidative enzymes that can seriously damage host tissues. We have previously demonstrated that neutrophil invasion and activation within the thrombus of human aortic abdominal aneurysms are the main biological impediments to the repair process that shifts the thrombus phenotype from a potential healing substrate to an aggressive neotissue.

One of the strengths of the study by Mangold et al13 resides in the use of human biological material. When compared with mice, in which the blood count is low in granulocytes (<20%), polymorphonuclear neutrophils are the predominant type of circulating leukocytes (50%–70%) in humans and are therefore systematically present in human thrombi. Concordantly, important ancillary results of Mangold et al13 highlight the fact that neutrophils and NETs are significant components of human coronary thrombi that do not only represent new therapeutic targets for thrombolysis but also provide a source of new biomarkers to define the biological process of acute coronary syndrome. In fact, the authors show that measurement of free double-stranded DNA can be used as a plasma biomarker of coronary thrombus NET burden, in addition to the more classical markers of neutrophil activation (neutrophil elastase and myeloperoxidase).
Because pathogens are one of the strongest triggers of NET formation\textsuperscript{7} and to explain the observed activation of neutrophils at the culprit lesion site, the authors explored the thrombus content in bacterial species from the buccodental microbiota. Their results confirm the high frequency of contamination of coronary thrombi by weak buccodental pathogens, such as Streptococcus species. Oral bacteria have been detected in carotid endarterectomy samples\textsuperscript{18,19} and in thrombectomy samples.\textsuperscript{20} If one considers the effect that bacteria can have on the deleterious biological activities of thrombi, one can imagine that thrombus contamination also fosters thrombus- and neutrophil-dependent injury in ST-elevation acute coronary syndrome. Human abdominal aortic aneurysms, intracerebral aneurysms, and infective endocarditis nicely illustrate how interactions between bacteria and the thrombus can fuel thrombus-dependent injury, notably by promoting neutrophil recruitment and activation.\textsuperscript{12,21}

Conclusions and Perspectives
Intravascular thrombi, whatever their initial cause and localization, are highly pathogenic because they not only induce downstream tissue ischemia by occluding arteries but also participate in injury of the surrounding tissue by releasing proteolytic and oxidative enzymes. Because of the various mechanisms of resistance to thrombolysis by rt-PA, new therapeutic approaches for the treatment of occlusive thrombosis are being actively investigated. In this context, the study of Mangold et al\textsuperscript{13} is of critical interest as it designates NETs as a source of new biomarkers to define the biological process of acute coronary syndrome. Human abdominal aortic aneurysms, their results confirm the high frequency of contamination of coronary thrombi by weak buccodental pathogens, such as Streptococcus species. Oral bacteria have been detected in carotid endarterectomy samples\textsuperscript{18,19} and in thrombectomy samples.\textsuperscript{20} If one considers the effect that bacteria can have on the deleterious biological activities of thrombi, one can imagine that thrombus contamination also fosters thrombus- and neutrophil-dependent injury in ST-elevation acute coronary syndrome. Human abdominal aortic aneurysms, intracerebral aneurysms, and infective endocarditis nicely illustrate how interactions between bacteria and the thrombus can fuel thrombus-dependent injury, notably by promoting neutrophil recruitment and activation.\textsuperscript{12,21}

Acknowledgments
We are indebted to Mary Osborne-Pellegrin and Richard Bayles for help in editing the article.

Sources of Funding
This study was supported by grants from Fondation de France (no. 2012-29500) and Fondation pour la Recherche Médicale.

Disclosures
None.

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Key Words: Editorials – blood platelets – hemoglobins – infection – leukocytes
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Circ Res. 2015;116:1107-1108
doi: 10.1161/CIRCRESAHA.115.306050

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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