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Commentary

Phosphatidylserine trapped in the net: A new therapeutic target for the ischemic stroke?

Federico Rodriguez Lucci[a], Mirta Schattner[b,*]

[a] Department of Neurology, Vascular Neurology Division, Institute of Neurological Research. FLENI, Buenos Aires, Argentina
[b] Laboratory of Experimental Thrombosis, Institute of Experimental Medicine-CONICET-National Academy of Medicine, Pacheco de Melo 3081, Buenos Aires, Argentina

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Despite the recent therapeutic advances, ischemic stroke remains one of the leading causes of death and permanent disability worldwide. Although the thrombus itself is the primary target of pharmacological thrombolysis, surprisingly, little is known about the composition of thrombi that causes ischemic stroke.

Neutrophil extracellular traps (NETs) have been implicated in thrombosis [1]. NETs are DNA fibers decorated with histones and microbicidal proteins and are recognised as an effective strategy of innate immune cells to fight against infections [2]. Besides their immunological role, NETs also contribute to both the venous and arterial thrombosis. NETs provide a scaffold for platelets and red blood cells that form a complex to promote the coagulation cascade. Therefore, the uncontrolled formation of NETs is increasingly accepted as another pathogenic mechanism of thrombotic events, not only during infectious diseases but also under sterile inflammatory conditions such as myocardial infarction, deep vein thrombosis, cancer-related thrombosis and stroke [1].

Currently, little is known about the presence of NETs in ischemic thrombus due to stroke. Studies in mice models and humans show that NETs are formed during an acute ischemic stroke (AIS) and that they are involved in brain-blood barrier damage as well as in the mechanical and pharmacological resistance of thrombolysis induced by tissue plasminogen activator [3].

The pathogenic mechanisms involved in vessel occlusion mediated by NETs formation during stroke were further described in this issue of EBioMedicine, by Zhou and colleagues [4]. The authors found increased levels of phosphatidylserine (PS) entrapped in the NETs, activated platelets, and PS + platelet microparticles (PMP) in the carotid lesion site (CLS) of patients with AIS. They also demonstrated that plasma from the CLS triggers PS exposure on neutrophil membranes and activates platelets that drive NETs formation. Moreover, the authors show that the DNA scaffold supports the binding of PS + PMP that together with the PS exposed on the NET allow binding of coagulation factors that catalyses thrombin generation and the formation of fibrin.

Although previous studies have shown that platelets promote the NET generation [5] and PMP expressing PS are involved in thrombus formation [6], this study reveals that the crosstalk between platelets, NETs, PMP and particularly PS in the local area of the carotid lesion might be critical for vessel occlusion.

It has been shown that NETs induce endothelial injury. However, most of the available information comes from animal models or in vitro studies using cells from healthy subjects [7,8]. Zhou et al., found that NETs from the CLS of patients with AIS trigger endothelial cell activation, increase their procoagulant activity and induce cell injury due to the proteolytic activity of metalloprotease-9 parenthesis should be deleted and replaced by metalloprotease-9 and elastase on endothelial cell junctions. While studies on endothelial cells are extremely important to understanding the pathophysiology of atherothrombotic diseases, it is essential to note that the study of Zhou et al., was performed using human umbilical endothelial cells which may not fully represent the in vivo situation.

Despite many advances in our understanding of ischemic stroke, cryptogenic strokes remain a diagnostic and therapeutic challenge. Atherothrombosis is a multifactorial process that involves the intricate participation of different cells and molecules. The authors describe a novel mechanism for hypercoagulability and acute thrombotic complications in AIS patients with carotid lesions, in which PS and NETs appear to have a destructive role.

Neutrophils have been traditionally recognized as major mediators of a deleterious inflammatory response in AIS, but their potential as a therapeutic target remains unexplored. Their ability to form NETs, recruit platelets and PMP through PS expression appears to be a novel and promising target for therapeutic intervention. However, several questions remain to be answered. What triggers NET formation in the microenvironment of the CLS? Neutrophils are not the only immune cell capable of triggering NET formation [9], so what could be the role of DNA traps released by other inflammatory cells during AIS? The levels of nuclear as well as mitochondrial DNA are increased in stroke patients [10]. We do not know yet whether both types of DNA traps exert similar prothrombotic and coagulation activities. Is the PS exposure on the NET an “eat me signal” for macrophages? Which risk factor

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* Corresponding author.
E-mail address: mschattner@hematologia.anm.edu.ar (M. Schattner).

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is more important to control in order to treat or prevent ischemic stroke: the formation of NETs, PS externalization, platelets and/or endothelial activation? One, two or a little of each factor? Embolic stroke of an unknown source is caused by embolic disease and associated with an elevated risk of recurrent ischemic strokes and clinically silent cerebral ischemic lesions. Are there any differences in NETs or PS expression between thrombi with different etiology?

These and many other questions are still yet to be answered. We look forward to more basic and clinical studies that would facilitate in solving this intriguing puzzle.

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