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Platelet-Derived MRP-14 Induces Monocyte Activation in Patients With Symptomatic Peripheral Artery Disease

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ABSTRACT

BACKGROUND Peripheral artery disease (PAD), a diffuse manifestation of atherothrombosis, is a major cardiovascular threat. Although platelets are primary mediators of atherothrombosis, their role in the pathogenesis of PAD remains unclear.

OBJECTIVES The authors sought to investigate the role of platelets in a cohort of symptomatic PAD.

METHODS The authors profiled platelet activity, mRNA, and effector roles in patients with symptomatic PAD and in healthy controls. Patients with PAD and carotid artery stenosis were recruited into ongoing studies (NCT02106429 and NCT01897103) investigating platelet activity, platelet RNA, and cardiovascular disease.

RESULTS Platelet RNA sequence profiling mapped a robust up-regulation of myeloid-related protein (MRP)-14 mRNA, a potent calcium binding protein heterodimer, in PAD. Circulating activated platelets were enriched with MRP-14 protein, which augmented the expression of the adhesion mediator, P-selectin, thereby promoting monocyte-platelet aggregates. Electron microscopy confirmed the firm interaction of platelets with monocytes in vitro and colocalization of macrophages with MRP-14 confirmed their cross talk in atherosclerotic manifestations of PAD in vivo. Platelet-derived MRP-14 was channeled to monocytes, thereby fueling their expression of key PAD lesional hallmarks and increasing their directed locomotion, which were both suppressed in the presence of antibody-mediated blockade. Circulating MRP-14 was heightened in the setting of PAD, significantly correlated with PAD severity, and was associated with incident limb events.

CONCLUSIONS The authors identified a heightened platelet activity profile and unraveled a novel immunomodulatory effector role of platelet-derived MRP-14 in reprograming monocyte activation in symptomatic PAD. (Platelet Activity in Vascular Surgery and Cardiovascular Events [PACE]; NCT02106429; and Platelet Activity in Vascular Surgery for Thrombosis and Bleeding [PIVOTAL]; NCT01897103) (J Am Coll Cardiol 2018;71:53-65)

Peripheral artery disease (PAD) is a clinical manifestation of systemic atherosclerosis provoking stenosis of the arteries supplying the lower limbs. It is estimated that more than 200 million people have PAD worldwide (1), and individuals with symptomatic PAD are at heightened risk for cardiovascular morbidity and mortality accompanied by impairment of quality of life (2). Although

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ABBREVIATIONS AND ACRONYMS

CAS = carotid artery stenosis

CLI = critical limb ischemia

LPA = leukocyte-platelet aggregate(s)

MACLE = major adverse cardiovascular and limb events

MPA = monocyte-platelet aggregate(s)

MRP = myeloid-related protein

PAD = peripheral artery disease

PR = platelet releasate

the pathogenesis of atherosclerosis affecting major coronary arteries is well characterized, the physiopathology of PAD, which manifests preferentially at lower extremity territories, is still unclear, and the mechanisms that regulate this complex disorder are not well understood.

Cumulative clinical and experimental studies have well established that platelets directly contribute to the development of atherothrombosis (3-5). Notably, antiplatelet therapies targeting thrombus formation are the mainstay for vascular occlusive diseases such as acute myocardial infarction and stroke. Although increased circulating platelet activity was previously described to correlate with the occurrence of PAD, knowledge of the mechanisms pertaining to this clinical observation is still lacking. Besides, thrombus-free atherosclerotic lesions causing stenosis also manifest in asymptomatic individuals with PAD, suggesting that in addition to their thrombotic potential, platelets can perform alternative sentinel functions in PAD. Coincidentally, the immunomodulatory role of platelets has been illuminated in early atherosclerosis (6-9). Because neither thrombosis nor rupture is allocated to lesions in their infancy, we can speculate that at this stage, when inflammation culminates, platelets can interact with key immune players to drive atherosclerosis. Indeed, being uniquely positioned in the peripheral blood, platelets can perform effector functions and act at the interface of the major inflammatory cell population by engaging different receptor-ligand bridges to direct the inflammatory response in tissues. As such, increased circulating platelet-leukocyte clusters have been demonstrated in patients with myocardial infarction, with angina, and during cardiopulmonary bypass (10). Recently, a deleterious role of platelet and neutrophil extracellular trap interaction was also described in sepsis (11).

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Pharmacological attempts have proven promising in disrupting such platelet-immune networks in different pathological settings (12). Collectively, these studies reinforce the role of activated platelet interactions in diseases, supplementary to their role in thrombus formation. However, so far, clinical evidence and functional evaluation of such platelet-immune complexes are completely undefined in the context of PAD.

Given that activated platelets are a major culprit in the pathogenesis of atherothrombosis and are prone to form interactions with circulating cellular subsets, we aimed to determine the effector role of platelets in a cohort of PAD. Here, we show that monocyte-platelet aggregates (MPA) were robustly increased in PAD and that myeloid-related protein (MRP)-14 harbored in activated platelets was instrumental in this process. Platelet-derived MRP-14 further activated monocytes by inducing the expression of proatherogenic markers and their directed migration. Moreover, MRP-14 was higher in PAD patients with critical limb ischemia (CLI) and was predictive of incident cardiovascular and limb events. Together, our present results identify a novel and central effector role for platelet-derived MRP-14 to synergize physically with activated monocytes and foster inflammation in PAD.

METHODS

Details of the experimental methods are available in the Online Appendix. Briefly, informed consent and recruitment were performed under the New York University Langone Medical Center Institutional Review Board. Patients with PAD and carotid artery stenosis (CAS) were recruited into a biorepository and ongoing studies (NCT02106429 and NCT01897103) investigating platelet activity.

Platelets were isolated by negative CD45 selection as previously described (13). To ensure purity of platelet population, only samples with low CD45 transcript levels (greater than 35 cycles) were used for RNA sequencing. In addition, only samples with platelet-leukocyte ratio of 1×10^7 were used for platelet transcriptomics (14,15). MPA were measured by double $CD61^+CD14^+$ staining and imaged using scanning electron microscopy.

STATISTICAL ANALYSIS. Data are reported as mean ± SEM or SD where appropriate. Clinical studies in which results were not normally distributed, median and interquartile range (first to third quartiles) were presented. Statistical significance between 2 experimental groups was performed using a parametric Student's *t*-test, nonparametric Mann-Whitney *U* test, or Spearman test for correlations datasets.

Conditional logistic regression was used to estimate relative risk and 95% confidence interval after the population was divided into groups on the basis of the median cutpoint for MRP-14. Adjusted risk estimates were obtained from regression models that adjusted for cardiovascular risk factors including age, sex, race/ethnicity, smoking status, and history of diabetes, hyperlipidemia, and coronary artery disease. All probability values were 2-tailed, and p values of <0.05 were considered statistically significant.