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Coronary clot composition after myocardial infarction: Thrombus age matters

Atherothrombosis, precipitated by the rupture of an atherosclerotic lesion and subsequent thrombus formation, is the major cause of acute coronary syndrome and cardiovascular death. While death rates due to acute myocardial infarction (MI) have dropped dramatically thanks to modern interventions including primary percutaneous coronary intervention, post-MI complications still lead to substantial morbidity and mortality in survivors. Life-threatening complications are recurrent thromboembolic events and adverse remodeling of the heart. A major driver of both types of adverse consequence is vascular inflammation, which creates a local pro-thrombotic milieu and promotes secondary injury and fibrotic remodeling of surrounding tissues. Fresh clots are characterised by prominent NADPH oxidase activity and lipid peroxidation [1], which will amplify oxidative injury upon reperfusion of the infarcted myocardium. Culprit cells transmitting such oxido-inflammatory signals are primarily monocyte-derived macrophages and neutrophil granulocytes. Seminal work some 15 years ago by Brinkmann et al [2] showed that neutrophils release fibrous web-like structures termed neutrophil extracellular traps (NETs), consisting of histone-associated DNA and microbicidal granular peptides and proteases. Depending on stimulus and context, the casting of NETs results in neutrophil rupture and death (suicidal NETosis), or nuclear blebbing and expulsion of DNA without immediate neutrophil death, leaving phagocytic functions intact (vital NETosis). Primary function of NETosis is to aid the capture and destruction of invading pathogens, and to limit collateral damage to host tissues by escaping neutrophil proteases. However, both forms of NETosis have also been intricately linked with immunothrombosis of various aetiologies [3,4]. Besides their potent capacity for platelet activation, NETs provide a structural scaffold for platelets, erythrocytes and plasma proteins like fibrinogen and von Willebrand factor (VWF), thereby stabilizing the clot and making it less susceptible to lysis [3,5,6]. NETs are moreover thought to contribute significantly to the local pro-oxidant and pro-inflammatory milieu within coronary thrombi of patients with ST-elevation myocardial infarction (STEMI) [1].

Neutrophils have been identified as the predominant source of such extracellular structures in early atherothrombotic plaques assessed at autopsy after MI [7]. Yet macrophages also contributed numerically significant amounts of "METs," indicating that neutrophils do not have exclusive rights to cast out such extracellular traps, or ETs. In intact plaques and late organised thrombi, macrophage-derived METs in fact predominated over neutrophil-derived NETs, while all thrombi also contain some ETs that were clearly attributable to mast cells and eosinophils. This has led to the idea that different leukocytes can undergo "ETosis" throughout all stages of thrombus progression and maturation, and that the cellular composition of the clot will, to a certain extent, determine the pro-inflammatory phenotype of the thrombus.

In this issue of the *International Journal of Cardiology & Heart and Vasculature*, Pertiwi and colleagues confirm that coronary aspirates from STEMI patients are consistently characterised by the presence of neutrophil and macrophage ETosis, regardless of thrombus age [8]. Assessment of ETosis rate, however, unveiled a temporal shift in the active participation of ET components with thrombus development. Fresh and lytic clots exhibit a high ETosis rate, while late, organised, clots containing more actively proliferating smooth muscle cells and endothelial cells showed subdued ETosis. Whether this is relates also to the switch from predominantly neutrophil etosis in early clots, to primarily macrophage-derived ET formation as the thrombus ages and reorganizes, as described above, requires further study. In any case, the observations by Pertiwi et al [8] fit with other reports indicating that clot-associated (N)ET formation and composition vary with thrombus age [9,10]. The clot provides a highly proteolytic environment [11], containing active coagulant proteases such as thrombin and plasmin, and the granular neutrophil proteases myeloperoxidase (MPO), neutrophil elastase (NE) and cathepsin G. Proteolysis of ET structures by entangled proteases will both alter their proteomic signature [12] and lead to their fragmentation [13]. Although ET dissolution with increasing thrombus age and re-organisation will abrogate the functional activity of some clot components, proteolytic fibrin degradation will also progressively loosen the thrombus itself. This will in turn allow active coagulation factors such as thrombin, and other oxido-inflammatory mediators, including ET components, to access surrounding cellular surfaces and induce persistent modifications of the local tissue architecture and properties.

It therefore appears that there are temporal differences in the way clot-associated ETs propagate thromboinflammatory signals into the myocardium, which raises many important questions. How can ETs cast within a wall-bound blood clot talk to the heart? A recent systematic assessment of the NET proteome identified over 160 different proteins [12], with main components being histones, MPO, NE, cathepsin G and lactoferrin. Each of these has the capacity to engage receptors on target cells within the clot or in the surrounding tissue, and thereby perpetuate inflammatory signals. Extracellular histones are prototypical damage-associated molecular patterns (DAMPs), eliciting sterile inflammatory signaling through activation of toll-like receptors (TLR) with subsequent activation of the NLRP3 inflammasome in immune cells and resi-

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dent tissue cells [14]. Further potent fibro-inflammatory triggers are provided by the engagement of cellular protease-activated receptors (PAR). These G-protein coupled receptors are activated by proteolytic cleavage in response to coagulant proteases such as thrombin or the neutrophil granular enzymes NE and cathepsin G, and contribute markedly to thromboinflammation and adverse tissue remodeling [15]. Neutrophil-secreted MPO generates highly reactive species such as acrolein and hypochlorous acid (HOCl) [5], which readily oxidise a range of thiol-containing proteins including serum albumin, glutathione, fibrinogen, thioredoxin and low-density lipoprotein. Depletion of front-line antioxidant defense proteins is therefore an immediate consequence of oxidative protein modification, but over the longer term, detrimental effects will also arise from binding of the modified proteins to scavenger receptors such as CD36. This may trigger DAMP-like actions that go beyond immediate adaptive responses to acute stress [16], and could initiate the unfolded protein response (UPR), since oxidatively modified proteins taken up by the cell are recognised by the proteostatic system as misfolded. Exhaustion of this quality control process then drives endoplasmic reticulum (ER) stress, which has been linked to cardiac injury and fibrotic remodeling after MI [17]. How much of this is directly attributable to leukocyte ETs escaping from the mural thrombus, however, remains to be assessed.

Balancing this apparently overwhelming inflammatory drive of clot-associated ETosis, several components found in neutrophil-derived ETs actually aid resolution and healing, such as neutrophil gelatinase-associated lipocalin (NGAL), calprotectin (S100A8/S100A9) and the cathelicidins [18]. To date, no study has shown how production and activity of these protective factors changes with thrombus age. Clearly, we require more detailed understanding of phenotype and function of clots [19] and clot-associated ETosis over time, in order to best tailor therapy after STEMI. Hitting ETs with a comprehensive pharmacological compound for destruction could potentially abolish their beneficial as well as their detrimental effects.

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