New insights into thromboinflammation through confocal intravital microscopy and thrombosis models

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Introduction: Microvascular thrombosis is associated with intensive inflammation, and thromboinflammatory responses are common in many pathological conditions including ischemia reperfusion (IR) injury and trauma. Despite the pathophysiological importance, the mechanisms by which platelet thrombi induce leukocyte recruitment and inflammatory responses remains poorly understood. This is mainly due to the lack of suitable intravital imaging technologies as well as suitable animal models of thrombosis.

Aim: To develop confocal intravital microscopy and thromboinflammation models to examine how platelet thrombi promote leukocyte recruitment.

Methods: Thrombi were formed by needle puncture of mesenteric veins and stabilized by local microinjection of platelet agonists. Spontaneous microvascular thrombi were also induced by IR gut injury. Leukocyte recruitment to sites of vascular injury and microvascular thrombi post IR injury were monitored using confocal and differential interference contrast intravital (DIC) microscopy.

Results: The needle injury model leads to highly reproducible thrombus formation and leukocyte recruitment. This model also offers the advantage to manipulate platelet activation and thrombus growth by allowing local microinjection of platelet agonists. This model in combination with confocal intravital microscopy has allowed several novel observations of leukocyte adhesive responses to thrombi. First, thrombi at sites of vessel injury are highly efficient at inducing leukocyte recruitment relative to inflamed endothelium. This efficient leukocyte recruitment is mediated by the 3D shape of thrombi, creating local low shear zones that favor leukocyte accumulation. Second, the recruited leukocytes undergo migration towards the thrombus core which is facilitated through the development of chemokine gradient within the thrombus. Third, prominent fibrin formation occurs at sites of vessel injury and the fibrin polymers function as a physical barrier, negatively regulating leukocyte migration to the thrombus core. Fourth, local injection of potent agonists leads to the formation of procoagulant phosphatidylserine (PS) expressing platelets, resulting in the ongoing leukocyte aggregate formation and detachment at sites of vessel injury. High speed confocal microscopy reveals that leukocytes actively drag PS+ve platelets from the thrombus surface, and can bridge adjacent adherent leukocytes promoting leukocyte aggregation, leading to vessel occlusion. Confocal intravital microscopy of intestine post IR injury reveals extensive thrombotic response throughout the microvasculature, and efficient leukocyte recruitment by these spontaneous thrombi.

Conclusion: The intravital confocal microscopy and thromboinflammation models have provided new insights into the complex platelet-leukocyte interaction and novel leukocyte responses. Their establishment has paved the way for the future investigation into the mechanisms regulating platelet-leukocyte interactions.