VISUALIZING EXCESSIVE LEUKOCYTE ACCUMULATION TO SITES OF ENDOTHELIAL INJURY

Imala Alwis, Zane Kaplan, Mehran Ghasemzadeh, Yuping Yuan, David Bark, Francisco Tovar-Lopez, Warwick Nesbitt, Shaun Jackson
Australian Centre for Blood Diseases (ACBD)
Monash University
89 Commercial Rd, Melbourne, Victoria 3004, Australia
E-mail: imala.alwis@monash.edu

Background: Ischemia-reperfusion injury is associated with an intense inflammatory infiltrate that exacerbates tissue injury. It is generally considered that ischemic injury to the endothelium, leading to upregulated expression of adhesion molecules and proinflammatory cytokines, is the principal mechanism promoting leukocyte recruitment and inflammation. A growing body of evidence has suggested a potentially important role for the proinflammatory function of platelets in exacerbating leukocyte recruitment, inflammation and tissue damage following I/R injury, however the mechanisms responsible for this remain poorly defined.

Methods: We established a murine model of intestinal I/R injury and performed intravital experiments using a Nikon A1R resonant scanning confocal microscope to analyze platelet and leukocyte adhesive responses in ischemic tissue. We subsequently developed a model of mechanical injury using micromanipulator needles in the mesenteric vasculature of mice to enable investigation of the mechanisms regulating platelet-leukocyte interactions. Particle image velocimetry (PIV) and computational fluid dynamics (CFD) were used to measure rheological parameters.

Results: Intravital confocal imaging of the murine intestinal microvasculature following I/R injury identified distinct patterns of leukocyte recruitment in the ischemic tissue, ranging from minimal stable adhesion to regions of extensive leukocyte accumulation and tissue infiltration. Significantly, the regions of exaggerated leukocyte adhesion correlated with the presence of platelet-rich thrombi, with leukocytes preferentially adhering to larger thrombi. Leukocytes adherent to thrombi had a polarised, elongated morphology with enhanced motility, suggesting a potentially important role for thrombi in promoting localized infiltration of leukocytes into ischemic tissue. To identify the mechanisms by which platelet thrombi mediate efficient leukocyte recruitment in vivo, we developed a localized model of microvascular injury using microinjector needles. In this model we controlled the level of platelet activation by microinjecting platelet agonists and identified thrombin as the most potent inducer of leukocyte-thrombus interactions. There was a direct correlation between thrombus size and the efficiency of leukocyte recruitment and computational fluid dynamic analysis (CFD) revealed that the 3-dimensional shape of the thrombus resulted in the formation of low shear pockets where leukocyte accumulation preferentially occurred. Notably, large thrombi and potent platelet stimulation were not sufficient to induce efficient leukocyte recruitment in arteries. However, controlled manipulation of arterial flow rates demonstrated that the bulk flow rate had a profound impact on leukocyte-thrombus interactions, with a ~80% reduction in arterial flow leading to a >50 fold increase in leukocyte recruitment.

Conclusion: Our ability to visualize leukocyte-thrombus accumulation enables us to identify the key biochemical and biophysical factors which modulate leukocyte-thrombus interactions in vivo. Our results define the cooperative influences of both biochemical (platelet activation status and thrombin generation) and biophysical factors (loco-regional rheological effects imparted by thrombus size and prevailing bulk flow) in regulating leukocyte recruitment by microvascular thrombi. These findings may help explain the potent thromboinflammatory response that is characteristic of I/R injury, where excess thrombin generation and platelet activation, in combination with disturbed blood flow amplifies the thromboinflammatory response.