

Conference Series LLC Joint International Event on
5th European Immunology & Innate Immunity
July 21-23, 2016 Berlin, Germany

C-type lectin-like receptor 2, P2Y12 receptor and $\beta 3$ integrin mediate bacterial DNA mimetic-induced platelet activation

Cecile Oury¹, Celine Delierneux¹, Alexandre Hego¹, Nathalie Donis¹, Christelle Lecut¹, Maud Vandereyken¹, Lucia Musumeci¹, Souad Rahmouni¹, Johannes A Eble², Jochen Schneider³ and Patrizio Lancellotti¹

¹University of Liege, Belgium

²University of Munster, Germany

³University of Luxembourg, Luxembourg

Short nuclease-resistant phosphorothioate synthetic CpG motif-bearing oligonucleotides (CpG ODNs) mimicking bacterial DNA display potent immunostimulatory activity. Pre-clinical studies and human clinical trials have demonstrated that CpG ODNs significantly improve vaccine-specific antibody responses. CpG ODN recognition by B-cells and plasmacytoid dendritic cells depends on the interaction of CpG ODNs with the intracellular Toll-like receptor 9 (TLR9). Besides immune cells, a recent study indicates that phosphorothioate modified CpG ODNs activate platelets and, therefore, may cause dangerous thrombotic events. In this work, we used pharmacological and genetic approaches on human and mouse platelets to investigate the mechanisms of platelet activation by CpG ODNs in order to identify potential protective strategies. We showed that CpG ODNs bound on platelet surface and were internalized. They caused P-selectin exposure and binding of fibrinogen and fibronectin on platelet surface and induced their aggregation. *In vivo*, intravenous injection of CpG ODN in mice promoted thrombus growth and fibrin generation at site of endothelial injury. Surprisingly, the use of TLR9-deficient platelets indicated that TLR9 signaling was dispensable for CpG ODN induced platelet activation. We found that CpG ODNs stimulated platelet tyrosine kinase-dependent pathway and Syk phosphorylation, similar to ITAM and $\alpha_{IIb}\beta_3$ integrin outside-in signaling. Mouse platelets deficient for the hemITAM C-type lectin-like receptor CLEC-2 were unable to capture and internalize CpG ODN. CLEC-2 deficiency abolished CpG ODN-induced platelet activation and aggregation. Accordingly, CpG ODN stimulated CLEC-2 dimerization in human platelets and CpG ODN-induced platelet aggregation was inhibited by an anti-CLEC-2 blocking antibody. Furthermore, $\beta 3$ -deficient platelets could not be activated by CpG ODNs. In contrast, mouse platelets deficient for the collagen ITAM receptor GPVI responded normally to CpG ODNs. Pre-treating platelets with the ATP/ADP scavenger apyrase or with the P2Y12 receptor antagonist cangrelor fully inhibited CpG ODN uptake and subsequent platelet activation, while the cyclooxygenase inhibitor indomethacin and the $\alpha_{IIb}\beta_3$ antagonist's tirofiban and eptifibatide conferred partial protection. In agreement with these results, CpG ODN failed to activate platelets from clopidogrel-treated patients or from a patient with Glanzmann thrombasthenia. In conclusion, CpG ODN-induced platelet activation depends on multiple platelet receptors including CLEC-2, P2Y12 and $\alpha_{IIb}\beta_3$. Importantly, inhibiting P2Y12 receptors with clinically available antiplatelet drugs may confer full protection against possible adverse pro-thrombotic effects of CpG ODN vaccine adjuvants.