**CLEC-2 is required for the activation of mouse platelets by bacterial DNA mimetics**

***Céline Delierneux,1 Alexandre Hego,1 Christelle Lecut,1*** *Maud Vandereyken,2 Lucia Musumeci,2 Souad Rahmouni,****2 Vincent Bours,3 Patrizio Lancellotti,1,4 Cécile Oury1***

1Laboratory of Thrombosis and Hemostasis, GIGA-Cardiovascular Sciences, University of

Liège, Liège, Belgium.

2Immunology and Infectious Diseases Unit, GIGA-Signal Transduction, University of Liège, Liège, Belgium.

3Department of Human Genetics, GIGA Research Center, University of Liège, Liège, Belgium

4University of Liège Hospital, GIGA Cardiovascular Sciences, Department of Cardiology, Heart Valve Clinic, CHU Sart-Tilman, Liège, Belgium.

**Background:** Short nuclease-resistant phosphorothioate synthetic CpG motif-bearing oligonucleotides (CpG ODNs) mimicking bacterial DNA display potent immunostimulatory activity and are therefore being used in clinical trials as vaccine adjuvants. Cellular uptake and activation depends on the interaction of CpG ODNs with the C-type lectin receptor DEC-205 and subsequent stimulation of the Toll-like receptor 9 (TLR9) and myeloid differentiation primary response 88 (MyD88) signaling cascade. Platelets express TLR9, MyD88, and the C*-*type lectin-likereceptor2 (CLEC-2). However, the impacts of CpG ODNs on platelet function have been elusive.

**Aims:** To evaluate whether CpG ODNs affect platelet activation and thrombus formation via CLEC-2 and TLR9.

**Methods:** We incubated washed platelets or whole blood from TLR9-, MyD88- or CLEC-2- deficient mice with CpG ODNs. We performed platelet aggregometry, flow cytometric binding and platelet activation assays as well as signal transduction analyses. Thrombus formation and fibrin generation were also analyzed by intravital microscopy in mouse microcirculation upon intravenous injection of CpG ODNs.

**Results:** We show that **CpG ODNs bind on platelet surface and are internalized. They** activate platelets and induce their aggregation. TLR9- or MyD88-deficient platelets aggregated normally in response to CpG ODN. Interestingly, platelets deficient for the C-type lectin receptor CLEC-2 were unable to capture and internalize CpG ODN. CLEC-2 deficiency abolished CpG ODN-induced platelet activation and aggregation. CpG ODN stimulated CLEC-2 dependent tyrosine kinase pathway and Syk phosphorylation. In vivo, intravenously injected CpG ODN interacted with platelets adhered to laser injured arteriolar endothelia and promoted fibrin generation and thrombus growth.

**Conclusion:** CLEC-2 mediates CpG ODN uptake and subsequent platelet activation, independently of TLR9, which may serve an important role in the interplay between platelets and immunity.

**Keywords :** platelet activation, CLEC-2, bacterial DNA mimetics, mouse models