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POSTER ABSTRACTS

113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

Circulating NETs Promote Platelet-TLR9 Dependent Acute Chest Syndrome in Sickle Cell Disease

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Acute chest syndrome (ACS), a type of acute lung injury is one of the primary reasons for mortality among Sickle Cell Disease (SCD) patients. Although new evidence suggests that circulating neutrophil extracellular traps (cNETs) promote lung injury in SCD by triggering occlusion of pulmonary arterioles by neutrophil-platelet-erythrocyte thrombo-inflammatory aggregates, the molecular mechanism underlying cNETs-dependent lung vaso-occlusion remains unknown. Surprisingly, platelets are one of the few cell types that express Toll-like receptor 9 (TLR9), a receptor for double-stranded DNA on the surface (rather than in endosomes), however, the role of platelet-TLR9 in promoting pulmonary thrombo-inflammation in SCD has never been studied. Using intravital (in vivo) lung microscopy in live SCD mice and imaging flow cytometry (in vitro) of SCD mice plasma, we show for the first time that lung vaso-occlusion and elevated levels of cNETs in knock-in humanized SCD mice challenged with 10 μmol/kg oxy-hemoglobin (oxy-Hb) is associated with significant upregulation of TLR9 surface expression in platelets. Importantly, TLR9 inhibition using a function blocking Ab led to significant reduction by ~6 and ~4 fold in the frequency and size of lung vaso-occlusions in SCD mice challenged with IV oxy-Hb. Identical to SCD mice, TLR9 surface expression was also significantly higher in SCD than control human platelets, and the expression was further upregulated following treatment of SCD patient platelet-rich-plasma (PRP) with oxy-Hb. Remarkably, elevated TLR9 expression in SCD patient platelets was concomitant to increased phosphorylation of both downstream TLR9 pathway effector tank-binding-kinase-1 (TBK-1) and the TBK-1 substrate interferon regulatory factor-3 (IRF3), which is the major transcription factor driving IFN-1 response. Taken together, our current findings suggest for the first time that activation of nucleic acid receptor TLR9 on the surface of platelets by cNETs contributes to lung vaso-occlusion and acute chest syndrome in SCD.

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