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ACTIVATED PLATELETS INDUCE THE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS VIA TOLL-LIKE RECEPTOR-4 AND P-SELECTIN GLYCOPROTEIN LIGAND-1

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Introduction. It is well-established that the interaction between platelets and neutrophils and the formation of neutrophil extracellular traps (NETs),induced by this interaction, are crucial for the development of numerous diseases, including atherosclerotic cardiovascular disease, cancer, as well as cancer-associated thrombosis (CAT). Despite growing evidence that supports the involvement of various platelet and neutrophil receptors and soluble factors in the pathophysiologic mechanisms underlying the above disease states, the entire spectrum of the pathophysiologic conditions and disease states in which the above interaction is involved has not yet been fully elucidated.

Aim. The aim of the present study was to investigate the role of toll-like receptor-4 (TLR-4) and P-selectin glycoprotein ligand-1 (PSGL-1) in the formation of NETs, induced by activated platelets.

Materials and Methods. Whole blood from apparently healthy volunteers was used to prepare washed platelet suspensions, adjusted to a platelet number of 250,000/μL, as well as to isolate neutrophils. Washed platelets were subsequently activated in an aggregometer with 0.2 U/mL thrombin and the resulting platelet pellet was separated from the supernatant by centrifugation at 1,500×g for 20min at room temperature. Neutrophil suspensions were then activated to generate NETs, with the platelet whole suspension, the platelet pellet or the platelet supernatant, in the presence or absence of 1.25 μg/mL anti-TLR-4 antibody or 1.25μg/mL anti-PSGL-1 antibody. The formation of NETs was determined with ELISA, quantitating DNA-myeloperoxidase (DNA-MPO) complexes.

Results. The platelet whole suspension, the platelet pellet and the platelet supernatant increased the formation of NETs by 210±30%, 32±14% and 132±15%, respectively ($p<0.05$ for all comparisons). In the presence of anti-TLR-4, the NET formation induced by the above platelet preparations was inhibited by 43±12%, 35±19% and 91±17%, respectively ($p<0.05$ for all comparisons), while in the presence of antiPSGL-1 the formation of NETs was reduced by 48±7%, 100±1% and 99±1%, respectively ($p<0.05$ for all comparisons).

Conclusions. The above results suggest that TLR-4 and PSGL-1 may play an important role in platelet-induced NET formation and in the pathophysiologic conditions in which NETs are involved, including cancer and CAT.