Online supplementary Material to Badrnya et al. “Platelets directly enhance neutrophil transmigration in response to oxidised low-density lipoprotein” (Thromb Haemost 2012; 108.4)

Material and methods

Activation of human neutrophils

Neutrophil activation of CD11b was determined by flow cytometry. Isolated neutrophils were added to freshly isolated platelets at a physiologic ratio of 1:20. After stimulation with oxLDL (100 µg/ml) for 10 min, cells were incubated for 20 min with anti CD11b (activated)-APC (BioLegend, San Diego, USA) following fixation with 1% formaldehyde and analysed by flow cytometry. Alternatively, buffy coat-enriched whole blood was pretreated with anti P-selectin antibody for 5 min and stimulated with oxLDL (100 µg/ml) for 30 min. After 20 min of incubation with anti CD11b (activated)-APC and fixation with 1% formaldehyde cells were analysed by flow cytometry.

Quantification of reactive oxygen species

Buffy coat-enriched whole blood was either pretreated for 5 min with anti P-selectin antibody (10 µg/ml; R&D Systems, Abingdon, United Kingdom) or left untreated, and stimulated with oxLDL (100 µg/ml) for 10 min at room temperature. After dihydrorhodamine 123 (DHR123) (10 µM; Molecular Probes, Invitrogen, Stockholm, Sweden) staining for 10 min at dark, cells were fixed (1% formaldehyde) and analysed by flow cytometry.

Results

Platelets enhanced oxLDL-induced neutrophil CD11b activation and ROS formation via direct cell to cell interactions

To assess whether platelet-neutrophil interaction in response to oxLDL also results in a more activated molecule pattern, we determined CD11b activation in freshly
isolated neutrophils exposed to oxLDL either in the presence or absence of platelets. Indeed, CD11b activation in neutrophils (supplemental Figure 1A) was significantly enhanced by oxLDL and further significantly amplified upon addition of platelets.

To determine whether direct cell-cell interactions or platelet-derived soluble mediators liberated in response to oxLDL contribute to increased CD11b activation in the presence of platelets, we analysed oxLDL-induced CD11b activation in the presence and absence of anti P-selectin antibody. Blockage of P-selectin significantly attenuated the percentage of CD11b activated neutrophils from 35.8% to 19.4% (supplemental Figure 1B), indicating that direct cell-cell interactions are mainly responsible for oxLDL-mediated effects.

Moreover, oxLDL effectively increased the generation of reactive oxygen species (ROS) by neutrophils which could be significantly reduced to almost basal levels by anti P-selectin antibody (supplemental Figure 1C).

These data indicate that neutrophil function is induced by oxLDL, further amplified in the presence of platelets and abolished by blockage of P-selectin, thus mainly mediated via direct cell-cell interactions.
Suppl. Figure 1: Modulation of neutrophil function in response to oxLDL by platelets. A) Neutrophil activation in the presence and absence of platelets after stimulation with oxLDL as determined by CD11b activation. B) Effect of anti P-selectin antibody (10 µg/ml) on neutrophil activation induced by oxLDL in buffy coat-enriched whole blood. C) Influence of anti P-selectin antibody on oxLDL-induced generation of ROS (DHR 123) by neutrophils; A-C) Final concentration of oxLDL: 100 µg/ml; means ± SD of 6 independent experiments; * p < 0.05; ** p < 0.01.