ImmunoTools *special* Award 2018

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Leukocyte-platelet aggregates as a new tool to predict thrombosis

In recent years, the traditional view of the haemostatic system as being regulated by platelet activation (primary haemostasis) and coagulation (secondary haemostasis) has been increasingly challenged by new evidence that activation of the immune system strongly influences blood coagulation and pathological thrombus formation.

Primary haemostasis starts with platelet adhesion to the extracellular matrix. Activated during this process, platelets change shape and release the contents of their granules. Additionally, activation of glycoprotein (GP)IIb/IIIa, and binding of fibrinogen or Von Willebrand factor to this receptor, cross-links platelets and contributes to thrombus stabilization.

Activated platelets form also aggregates with monocytes and neutrophils via engagement of platelet surface P-selectin (released from the alpha granules) with leukocyte surface P-selectin glycoprotein ligand-1 (PSGL-1) which is constitutively expressed on the leukocyte surface. Leukocyte-platelet aggregates are stabilized by the binding of leukocyte macrophage-1 antigen (Mac-1) and platelet GPIbα, and to a lesser extent by leukocyte function-associated antigen 1 on leukocytes and intercellular adhesion molecule 2 on platelets. The resultant intracellular signalling causes the leukocyte surface expression of tissue factor (the main trigger of coagulation, normally absent from circulating blood) and the binding of FXa and/or fibrinogen to activated Mac-1. In this way, the leukocyte cell surface can provide a site for activation of coagulation. Coagulation is initiated by the activation of FVII by tissue factor (TF) and the formed TF-FVIIa complex converts FX to FXa. FXa produces the first traces of thrombin (FIIa), which in turn activates FV. FVa combines with FXa, increasing its efficiency by a 1000-fold, causing a burst of IIa formation. Apart from these, some other coagulation factors recruited on phospholipids also contribute to the formation of FIIa. In the final step, FIIa cleaves fibrinogen, leading to the formation of a fibrin network.

Excess platelet activation is associated with thrombotic disorders. P-selectin is considered as the ‘gold standard’ marker of platelet activation, however, *in vivo* circulating degranulated platelets rapidly lose their surface P-selectin. Upon activation, platelets also interact with leukocytes and it has been demonstrated that

circulating leukocyte-platelet aggregates are a more sensitive marker for *in vivo* platelet function. Circulating leukocyte-platelet aggregates are increased in stable coronary artery disease, unstable angina, acute myocardial infarction and cardiopulmonary bypass.

So to find out whether leukocyte-platelet aggregates could be used as a tool to predict thrombosis, an assay will be developed to measure monocyte-platelet aggregates and neutrophil-platelet aggregates. Citrate anti-coagulated whole blood will be used and incubated with a platelet agonist (CRP, ADP or TRAP), and with fluorescent conjugated antibodies against leukocytes (CD45, CD14, CD63, CD3, CD19) and platelets (CD61, CD42a, CD42b, CD62). A flow cytometer will be used to analyse leukocyte subgroups which can be identified based on specific fluorescence, forward and side scatter. These populations can then be further gated into platelet marker-positive (platelet-bound) and platelet marker-negative (platelet-free). The antibodies from Immunotools would greatly contribute in distinguishing these different populations.

ImmunoTools *special* AWARD for **Li Li** includes 25 reagents

APC – conjugated anti-human CD14, CD41a, CD42b, CD45, CD61, CD62P, CD63, Annexin V

FITC - conjugated anti-human CD14, CD41a, CD42b, CD45, CD61, CD62P, Control-Ig G1, Annexin V

PE - conjugated anti-human CD14, CD19, CD41a, CD42b, CD45, CD61, CD62P, Annexin V

PerCP - conjugated anti-human CD3

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