ImmunoTools *special* Award 2014

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Role of platelets in leukocyte activation and polarization

Platelets are anucleate blood cells that fulfill a central function in haemostasis by facilitating the cessation of bleeding. Platelets also play a pivotal role in inflammation, as they can modulate inflammatory reactions and immune responses by activation-dependent expression of surface receptors. Thereby platelets mediate the interaction with different leukocyte populations and modulate their activation and their function. Platelet-leukocyte interactions are an intended process to facilitate leukocyte migration into perivascular tissues. However, platelet-leukocyte interactions also occur in circulating blood, resulting in exacerbating tissue injury, which contributes to the clinical signs of various inflammatory diseases.

Interactions between platelets and leukocytes not only occur directly by cell-cell contact, but also by the release of a pleithora of soluble mediators and cytokines which are stored in granules within the platelet. α-Granules represent the most abundant type of granules and contain various preformed proteins, including coagulation factors, growth factors and pro-inflammatory mediators like platelet factor 4 (CXCL4/PF4), RANTES (CCL5), MCP-1 (CCL2), MIP-1α (CCL3), SDF-1α/CSCL12 and CD40L, all of which are known to act on monocytes.

At the present moment, it is still unknown how platelets are able to regulate the preferential release of individual mediators from granules upon activation with specific agonists to induce haemostatic or non-haemostatic responses. One explanation could be unequal distribution of proteins in the granules. Signaling via PAR-1, P2Y1/P2Y12 and GPVI was reported to favor proangiogenic factor release (e.g. SDF-1α, VEGF), whereas PAR4 signaling promotes an antiangiogenic response (e.g. PF4, endostatin).

In the present study we aim to further clarify the molecular crosstalk between leukocytes and platelets in both human and murine settings. In particular, we want to identify the proinflammatory markers released from platelet granules in response to different agonists and how they influence the activation of monocytes and their differentiation to macrophages as well as the polarization of macrophages towards M1 or M2 phenotype. In the first part of the study we will perform *in vitro* studies to examine the role of direct platelet-leukocyte interactions. Therefore we will activate isolated platelets with several distinct agonists and co-culture them with leukocytes. We will investigate the activation status of both platelets and leukocytes through measuring expression of activation markers on the cell surface as well as the formation of platelet-leukocyte aggregates by flow cytometry. To that end we will use

FACS antibodies identifying platelets (CD61) respectively leukocyte populations (CD45, Gr-1, CD14, CD16, CD11b, CD11c) as well as activation markers (CD62P, Annexin V, CD63, CD11b activated). Furthermore, we intend to explore the impact of platelet-derived inflammatory cytokines and chemokines by analyzing platelet releasate and its effect on leukocytes. The relevance of secreted factors will be validated by stimulating leukocytes with recombinant proteins (CXCL4/PF4, RANTES/CCL5, sCD40L and SDF-1α/CXCK12a).

In the second part of the study we aim to clarify the effect of platelet on the polarization of macrophages in the *in vitro* model. Direct interaction or the release of soluble mediators may modulate the polarization of macrophages toward M1 or M2 macrophages. Macrophage phenotype will be assayed on RNA (qRT-PCR) and protein level (FACS) using specific markers (CD80, CD86, CD163, CD206). In order to identify the effect of platelets on the switch of macrophages from M2 to the more inflammatory M1 phenotype we will polarize isolated macrophages towards M2 with rIL-4, rIL-13 and rIL-10 before assessing the effect of platelets or their releasate. GM-CSF and M-CSF treatment will serve as positive controls for the polarization towards M1 respectively M2 phenotype.

Platelet-leukocyte interactions mediate both positive and negative effect in inflammatory diseases by contributing to leukocyte recruitment as well as potentiating the inflammatory response. Thus, investigating the molecular interplay between platelets and leukocytes is of great interest and the service provided by ImmunoTools will be an essential contribution to our research.

ImmunoTools *special* AWARD for **Waltraud Schrottmaier** includes 24 reagents

FITC - conjugated anti-human CD86,

PE - conjugated anti-human CD11b, CD80,

PerCP - conjugated anti-human CD45,

APC -conjugated anti-human CD11c, Annexin V,

recombinant human cytokines rh GM-CSF, rh IL-4, rh IL-13, rh M-CSF, rh PF4v1/CXCL4V1, rh RANTES / CCL5, rh sCD40L / CD154, rh SDF-1α / CXCL12a,

PE - conjugated anti-mouse CD11b,

APC -conjugated anti-mouse CD45,

recombinant mouse cytokines rm GM-CSF, rm IL-4, rm IL-10, rm IL-13, rm M-CSF, rm RANTES / CCL5, rm sCD40L / CD154, rm SDF-1α / CXCL12a, DETAILS