ImmunoTools *special* Award 2017

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Regulation of leukocyte adhesion by integrin phosphorylation

Leukocyte adhesion to other cells and to the extracellular matrix is of critical importance for immune system function and has to be strictly regulated. Important molecules for leukocyte adhesion are selectins and the leukocyte-specific β2-integrins, which include LFA-1 (αL/β2), Mac-1 (αM/β2), αX/β2 and αD/β2. In addition, leukocytes contain several β1-integrins, among them the α4/β1 integrin (VLA-4). The Leukocyte adhesion deficiency disease, LAD, is caused by mutations in β2-integrins or integrin binding protein kindlin-3, which cause immunodeficiency resulting in recurrent infections.

We are interested in how leukocyte adhesion and migration are regulated by β1 and β2 integrins and integrin-associated proteins. In resting leukocytes, integrins are maintained in an inactive state. Activation of the integrins leads to conformational changes, which enables extracellular ligand binding but also activation of intracellular signalling pathways. We focus on how the LFA-1 integrin is activated to bind ligand or activating antibodies, and how this translates into various phosphorylation patterns on the cytoplasmic tails. These phosphorylations affect the association of proteins to the integrin cytoplasmic tail and initiate intracellular signalling, which result in cytoskeletal reorganization, changes in transcription and crosstalk to other integrins in the same cell, mediating their activity.

We have previously identified a signalling pathway in T cells from the T758-phosphorylated β2-chain, through binding of 14-3-3 and the Rac GEF Tiam1, actin cytoskeleton rearrangements by Rac1 and crosstalk to the β1/α4 integrin. The crosstalk changes the phosphorylation pattern of the β1/α4 integrin, the protein complexes formed with β1/α4 and consequently its activity. We continue characterizing the details of these signalling pathways and are looking for means to affect them through antibodies and small molecules.

We are also interested in how αL-chain phosphorylation changes integrin subcellular localization, complex formation and ultimately adhesion and migration. We aim to purify adhesion complexes from differently activated T cells, for which we would use

ImmunoTools chemokines, e.g. SDF1-alpha or RANTES, or activation through the T-cell receptor with anti-CD3. We identify the activation status of the integrin with specific activation-dependent antibodies through western blotting or flow cytometry using ImmunoTools fluorescently labelled antibodies and analyse binding proteins with antibodies through co-immunoprecipitations and mass-spec analysis.

We are further studying integrin-mediated processes in the bran immune cells, microglia. For these projects we use mouse cells from knockout animals. To verify the identity of the primary cell cultures and to activate the cells we would use mouse chemokines and antibodies from ImmunoTools.

ImmunoTools *special* AWARD for **Mikaela Grönholm** includes 24 reagents

anti-human antibodies for flow cytometry:

PE - conjugated anti-human CD11a, CD11b, CD11c, Control-IgG1

APC - conjugated anti-human CD11a, CD18, CD29, Control IgG1

recombinant human cytokines
rh IL-2, rh RANTES / CCL5, rh SDF-1α / CXCL12a

Annexin V for coating

anti-mouse antibodies for flow cytometry:

FITC - conjugated anti-mouse CD18, CD29, isotype control IgG2b

PE - conjugated anti-mouse CD11b, CD54, isotype control IgG2b

APC - conjugated anti-mouse CD11a, CD45, CD49d, isotype control IgG2b

recombinant mouse cytokines: rm MCP1 / CCL2, rm RANTES / CCL5

DETAILS more AWARDS