Laboratory of Leukocyte Signalling

Signalling by leukocyte surface receptors, Csk-binding proteins, relationship between signalling and leukocyte-driven pathologies

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The Laboratory of Leukocyte Signalling is studying the molecular mechanisms of signal transduction in leukocytes. Our interest has recently been focused on the interplay between adaptor proteins, Src-family kinases, and related kinase Csk. In addition, we are also involved in the research aiming at uncovering the relationship between signal transduction and leukocyte-driven pathologies. Src-family kinases are tightly controlled enzymes critically involved in the signalling via a number of leukocyte surface receptors such as T-cell and B-cell receptors for antigens. The majority of Src-family kinases are directly associated with cellular membranes through specific sequence motifs at their N-termini. Csk is a major negative regulator of these kinases. However, it lacks any membrane targeting sequences, and therefore it relies on the interactions with membrane-associated adaptor proteins to gain access to Src-family kinases and to efficiently regulate their activity. In the past, we discovered several novel members of this group of adaptor proteins and now we are working on the characterization of their functional a biochemical features. These studies include analysis of transmembrane adaptor termed SCIMP, which interacts with both positive (SLP65/76) and negative (Csk) regulators of signalling. We found that in B cells it is involved in MHCII-dependent reverse signalling and currently we are characterizing its role in the signalling pathways of the receptor for pathogenic fungi Dectin-1 in dendritic cells. Another protein that was discovered during our search for novel Csk-binding proteins is known as PSTPIP2. Importantly, defects in PSTPIP2 expression in mice result in an autoinflammatory disorder characterized by sterile inflammation of bones and skin, which closely resembles the human disease known as chronic recurrent multifocal osteomyelitis. We are now exploring the mechanisms of how Csk and lipid phosphatase SHIP1 (an additional inhibitory enzyme recently identified in our laboratory as a binding partner of PSTPIP2) contribute to the control of inflammation by PSTPIP2. Additional clinically relevant projects include studies of signalling proteins aberrantly expressed in childhood leukaemias (OPAL1) and research on changes in leukocyte signal transduction in patients with common variable immunodeficiency (CVID), both running in collaboration with clinical laboratories.

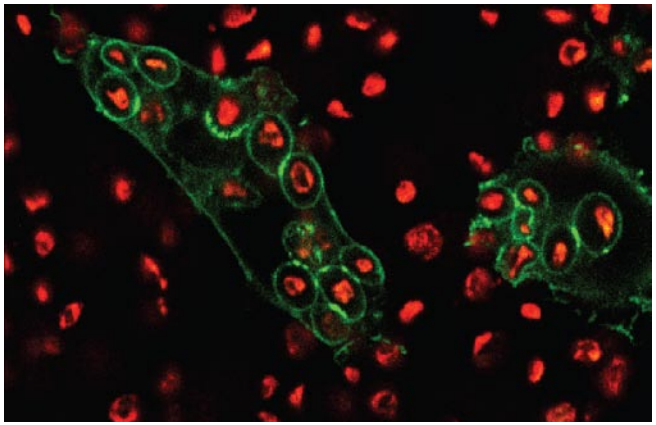

Fig. 3 SCIMP localization in dendritic cells during phagocytosis of Zymosan particles. Phagocytic uptake of Zymosan (consisting of yeast cell wall components) is mediated by Dectin-1 receptor. This image shows localization of SCIMP-EGFP (green) in dendritic cells engulfing Zymosan particles (red). Note the abundant presence of SCIMP in the membranes of Zymosan-containing phagosomes.

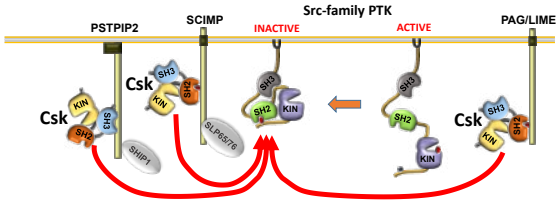

Fig. 1. Schematic representation of the regulation of Src-family kinases by Csk-binding proteins. Csk is recruited to the proximity of Src-family kinases by membrane adaptors such as SCIMP, PSTPIP2, PAG or LIME and then phosphorylates negative regulatory tyrosine at the C-terminus of Src-family kinases. This phosphorylation facilitates transition of Src-family kinases to the closed inactive conformation.

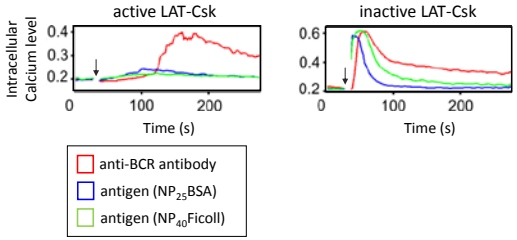

Fig. 2. One of the early outcomes of B-cell antigen receptor (BCR) stimulation is a rapid increase in intracellular calcium concentration. This image shows the effect of forced membrane targeting of Csk on this type of calcium response. Csk was targeted directly to the plasma membrane via fusion with the extracellular and transmembrane domain of transmembrane adaptor protein LAT (LAT-Csk). The left panel shows strongly reduced and delayed calcium response in the presence of membrane-targeted Csk (compare to the right panel where LAT-Csk activity was abolished by point mutation in the Csk kinase domain). The arrow points to the time when the stimulus was added to the sample.

- GACR, P305/11/0459 – The effect of transmembrane domains of integral proteins on dynamic organization of the plasma membrane of T lymphocytes, 2011-2014, T. Brdička
- GACR, P302/12/1712 – Function of Csk-anchoring proteins SCIMP and PSTPIP2 in leukocyte signalling and inflammation, 2012-2015, T. Brdička
- IGA MH, NT13271 – Phenotyping B- and T-cells in immunodeficiency, 2012-2015, T. Brdička

1. Liss A, Ooi CH, Zjablovskaja P, Benoukraf T, Radomska HS, Ju C, Wu M, Balaštík M, Delwel R, Brdička T, Tan P, Tenen DG, Alberich-Jorda M: The gene signature in CCAAT-enhancer-binding protein α dysfunctional acute myeloid leukemia predicts responsiveness to histone deacetylase inhibitors. **Haematologica 2013**.
2. Stepanek O, Draber P, Drobek A, Horejsi V, Brdicka T: Nonredundant roles of SRC-family kinases and syk in the initiation of B-cell antigen receptor signaling. **J Immunol 2013** 190(4): 1807-18.

From the left: Aleš Drobek, MSc / PhD Student, Jarmila Králová, MSc / PhD Student, Tomáš Brdička, PhD / Head of Laboratory, Daniela Glatzová, MSc / PhD Student (since 2014), Matej Fabišik, MSc / PhD Student (since 2014)

Not in the picture: Klára Kotlabová, BSc / diploma student (until 2013), István Dányi, MSc / PhD Student (until 2013)