Univerzita Karlova v Praze

Přírodovědecká fakulta

Studijní program: Imunologie

Mgr. Peter Dráber

Mechanisms of signal transduction by leukocyte surface receptors and transmembrane adaptor proteins

Mechanismy signální transdukce povrchovými receptory a transmembránovými adaptory leukocytů

Doktorská dizertační práce

Školitel:

Mgr. Tomáš Brdička, PhD

Laboratoř molekulární imunologie

Ústav molekulární genetiky, AV ČR

Praha, 2011

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 21. 9. 2011

Acknowledgement

I would like to thank to all the people who have collaborated on the publications presented in this thesis, especially to my supervisor Dr. Tomáš Brdička for his guidance and valuable help, to the head of the Laboratory of Molecular Immunology Prof. Václav Hořejši for the opportunity to work in a very pleasant environment and to my laboratory colleagues for creating a friendly and stimulating atmosphere.

1 Content

2 Abstract

The central role in the initiation and maintenance of the adaptive immune response is played by interaction of T cells with antigen presenting cells. Upon recognition of peptide-loaded MHC glycoproteins on the surface of antigen presenting cells by specific T cell, immunological synapse is formed and signaling events are initiated in both cells involved. The signal propagation is regulated by various molecules that can have positive or negative function, or even both, depending on specific circumstances. In this work we focused our attention on three topics all encompassing processes of signal propagation or regulation. First, we extended our understanding of the signaling processes taking place in the immunological synapse by the discovery of a new transmembrane adaptor protein SCIMP expressed on antigen presenting cells. Detailed study of this protein demonstrated that it is a positive regulator of MHCII signaling. Next two projects were focused on T cells. We described the role of another transmembrane adaptor protein termed PRR7 in the regulation of apoptosis and T cell receptor (TCR) mediated signaling in T cells. Finally, we provided evidence that in human T cells CD148 phosphatase can have both activatory and inhibitory effect on T cell receptor signaling.

Presented thesis consists of three publications :

1. Draber P, Vonkova I, Stepanek O, Hrdinka M, Kucova M, Skopcova T, Otahal P, Angelisova P, Horejsi V, Yeung M, Weiss A, Brdicka T. 2011. **SCIMP: transmembrane adaptor protein involved in MHCII signaling.** *Moll Cell Biol* [published ahead of print on 19 September 2011, doi:10.1128/MCB.05817-11]

2. Hrdinka M, Draber P, Stepanek O, Ormsby T, Otahal P, Angelisova P, Brdicka T, Paces J, Horejsi V, Drbal K. 2011. **PRR7 is a transmembrane adaptor protein expressed in activated T cells involved in regulation of T cell receptor signaling and apoptosis.** *J Biol Chem* 286: 19617-29

3. Stepanek O, Kalina T, Draber P, Skopcova T, Svojgr K, Angelisova P, Horejsi V, Weiss A, Brdicka T. 2011. **Regulation of Src family kinases involved in T cell receptor signaling by protein-tyrosine phosphatase CD148.** *J Biol Chem* 286: 22101-12

3 Abstrakt

Interakce T buněk s buňkami prezentujícími antigen hraje zásadní úlohu při zahájení a koordinaci adaptivní imunitní odpovědi. Poté, co T buňka rozpozná MHC glykoproteiny s navázaným antigenním peptidem na povrchu antigen prezentující buňky, dochází k tvorbě imunologické synapse a jsou spuštěny signální dráhy v obou zúčastněných buňkách. Aktivita signálních drah je regulována různými molekulami, které mají pozitivní nebo negativní efekt. Aktivity některých proteinů mohou mít i duální charakter v závislosti na dané situaci. V této práci jsme se zaměřili na tři témata týkající se regulace nebo propagace signálních drah. V první části jsme rozšířili znalost signálních dějů probíhajících v oblasti imunologické synapse, neboť jsme objevili nový transmembránový adaptorový protein SCIMP, který je exprimovaný na buňkách prezentujících antigen. Po důkladném studiu tohoto proteinu jsme prokázali, že se jedná o pozitivní regulátor signalizace přes MHCII glykoproteiny. Další dva projekty se týkaly T buněk. Nejprve jsme popsali význam dalšího transmembránového adaptorového proteinu nazvaného PRR7 v regulaci apoptózy a v signalizaci přes T buněčný receptor. Nakonec jsme se zaměřili na studium exprese fosfatázy CD148 v lidských T buňkách a prokázali jsme, že může mít aktivační i inhibiční efekt na signalizaci přes T buněčný receptor.

Základem předkládané disertační práce jsou tři publikace:

1. Draber P, Vonkova I, Stepanek O, Hrdinka M, Kucova M, Skopcova T, Otahal P, Angelisova P, Horejsi V, Yeung M, Weiss A, Brdicka T. 2011. **SCIMP: transmembrane adaptor protein involved in MHCII signaling.** *Moll Cell Biol* [published ahead of print on 19 September 2011, doi:10.1128/MCB.05817-11]

2. Hrdinka M, Draber P, Stepanek O, Ormsby T, Otahal P, Angelisova P, Brdicka T, Paces J, Horejsi V, Drbal K. 2011. **PRR7 is a transmembrane adaptor protein expressed in activated T cells involved in regulation of T cell receptor signaling and apoptosis.** *J Biol Chem* 286: 19617-29

3. Stepanek O, Kalina T, Draber P, Skopcova T, Svojgr K, Angelisova P, Horejsi V, Weiss A, Brdicka T. 2011. **Regulation of Src family kinases involved in T cell receptor signaling by protein-tyrosine phosphatase CD148.** *J Biol Chem* 286: 22101-12

4 Introduction

4.1 Antigen presentation to T cells

T cells are the central cells of the adaptive immune system, since they orchestrate and coordinate immune responses. The outcome of their action, if working properly, is the defense and long lasting protection against particular pathogens and also the surveillance of body tissues and prevention of cancer outbreak. Failure of T cell function results in profound defects varying from chronic or fatal infections to allergies, hypersensitivities, and autoimmune diseases.

By their specific T cell receptor (TCR), T cells recognize antigenic peptides presented by various cells on major histocompatibility complex class I or II glycoproteins (MHCI, MHCII). The ability of T cells to recognize MHCI or II is restricted via the expression of co-stimulatory molecules CD4 or CD8, respectively, on their surface. The process of antigen presentation leads to the activation of naive T cells by professional antigen presenting cells (APCs) and their differentiation into effector T cells. These effectors can subsequently both activate and coordinate different cells of the immune system to combat the pathogens (in the case of CD4+ T cells) or directly kill the infected target cells (in the case of CD8+ T cells).

Dendritic cells, monocytes, macrophages and B cells (1, 2) are all considered professional APCs. Dendritic cells and macrophages recognize and acquire pathogens by various surface receptors, such as scavenger receptors, complement or Fc receptors. B cells are able to recognize and engulf pathogens following recognition by specific B cell receptor (BCR). Naive T cells are most efficiently activated by dendritic cells. Under some circumstances, macrophages and B cells can also be responsible for this process. Later in the immune response, effector CD4+ T cells recognize antigen presented on the surface of macrophages and B cells and activate these cells. As a result macrophages become more effective in killing of engulfed pathogens and B cells with BCR specific for particular pathogen enter into the cycle of affinity maturation, leading to the production of high affinity antibodies.

Process of antigen presentation is accompanied by the formation of immunological synapse (IS) at the APC - T cell interface (3). Since the first observation of IS it has become clear that it is composed of several distinct regions, creating structure resembling “bull´s eye“. At the center of IS is an area known as central supramolecular activation cluster (cSMAC), containg pairs of TCR-MHC, coreceptors CD4 and CD8 and several signaling molecules, such as Src family kinases. Around cSMAC is an area called peripheral SMAC (pSMAC) containing pairs of adhesion molecules such as CD2-CD58 or LFA1-ICAM1 or LFA1-

ICAM3. Finally distal SMAC (dSMAC) contains molecules with large extracellular domains like CD45 (4).

Recognition of peptide-loaded MHC by TCR leads to various signaling events in both cells involved that will be described in the next sections.

4.2 TCR induced signaling pathways

Signaling pathways in T cells are well studied (Fig. 1). Upon TCR activation, Src family kinases Lck and Fyn phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) present in TCR associated CD3 subunits and ζ chains. Phosphorylated ITAMs subsequently serve as binding sites for Syk family kinase ZAP-70.

ZAP-70 is a cytoplasmic molecule containing two Src homology 2 (SH2) domains followed by a kinase domain at the C-terminus. In non-activated cells, ZAP-70 is held in autoinhibited closed conformation (5). Binding of ZAP-70 via its SH2 domains to phosphorylated ITAMs, induces a structural change in ZAP-70 resulting in the release of its kinase domain followed by stabilization of the open conformation via phosphorylation of tyrosines Y315 and Y319 by Lck or by another active ZAP-70 molecule (6). Once ZAP-70 is docked in active conformation at the membrane, it phosphorylates critical adaptor protein - linker for activation of T cells (LAT).

LAT is a member of the family of transmembrane adaptor proteins (TRAPs), characterized by a short extracellular part, a transmembrane domain followed by a palmitoylation motif and an intracellular part containing several protein-protein interaction motifs. Upon tyrosine phosphorylation, LAT functions as a scaffold molecule that binds to Grb2-related adaptor downstream of Shc (GADS). GADS forms a stable complex with Src homology 2 domain-containing leukocyte protein of 76 kDa (SLP76) and thus brings cytoplasmic adaptor SLP76 to the plasma membrane, where it becomes phosphorylated by ZAP-70. Subsequently phospholipase Cγ1 (PLCγ1) binds to LAT-GADS-SLP76 complex together with the member of Tec family kinases called IL2-inducible T-cell kinase (Itk) (7-9). Itk phosphorylates and activates PLCγ1, which subsequently cleaves membrane lipid fosfatidylinositol-4,5-bisphosphate (PIP_2) to cytosolic inositol-1,4,5-trisphosphate (IP_3) and membrane attached diacylglycerol (DAG), each of them serving as a second messenger.

Increase in the concentration of cytoplasmic IP_3 leads to the activation of calcium channels present on endoplasmic reticulum (ER), promoting release of calcium from ER to the cytoplasm. This event promotes opening of plasma membrane localized calcium-release

activated calcium (CRAC) channels, further increasing intracellular calcium concentration (10). The mechanism of this process, called store-operated calcium entry (SOCE), has been elucidated by the discovery that ER localized protein stromal interacting molecule 1 (STIM1) functions as a calcium sensor (11, 12). Decrease in calcium concentration in ER enables aggregation of STIM1 under the plasma membrane and this is then detected by the subunit of CRAC chanel ORAI1 (13, 14). Increase in cytoplasmic calcium concentration subsequently turns on the serine/threonine phosphatase calcineurin. Calcineurin dephosphorylates the transcription factor nuclear factor of activated T-cells (NF-AT), which is then translocated into the nucleus and initiates transcription of various genes (15).

Pathway initiated by the formation of DAG leads to the activation of transcription factor nuclear factor κB (NF-κB). NF-κB is held inactive in the cytoplasm by binding to inhibitor of NF-κB (IκB). The formation of DAG enables recruitment of protein kinase C-θ (PKCθ) to the plasma membrane and its activation. PKCθ then orchestrates formation of trimolecular complex CARMA1-BCL10-MALT1 (termed CBM). CBM promotes polyubiquitination and degradation of regulatory subunit NEMO of IκB kinase (IKK). This step releases active IKK that subsequently phosphorylates IκB, marking it for polyubiquitination and degradation in the proteasome. This releases active NF-κB, which may translocate into the nucleus and initiate transcription of various genes (16, 17).

Moreover, DAG also promotes membrane recruitement and PKCθ mediated activation of Ras guanyl nucleotide releasing protein (RasGRP) (18). RasGRP functions as guanyl nucleotide exchange factor (GEF) for small GTPase Ras (19, 20). Active Ras then initiates signaling cascade leading to the activation of mitogen-activated protein kinases (MAPK) extracellular signal-regulated kinase (Erk). Activated MAPK can subsequently phosphorylate and regulate function of various proteins, including subunits of transcription factor AP1 (21, 22).

Additional signaling pathway leading to Ras activation is mediated by binding of protein growth-factor-receptor-bound protein 2 (GRB2) to LAT. GRB2 interacts with Ras GEF called Son of sevenless homolog 1 (SOS1), and therefore brings SOS1 to the plasma membrane, where it mediates exchange of GDP for GTP on the membrane bound Ras, again leading to the initiation of MAPK Erk pathway (23).

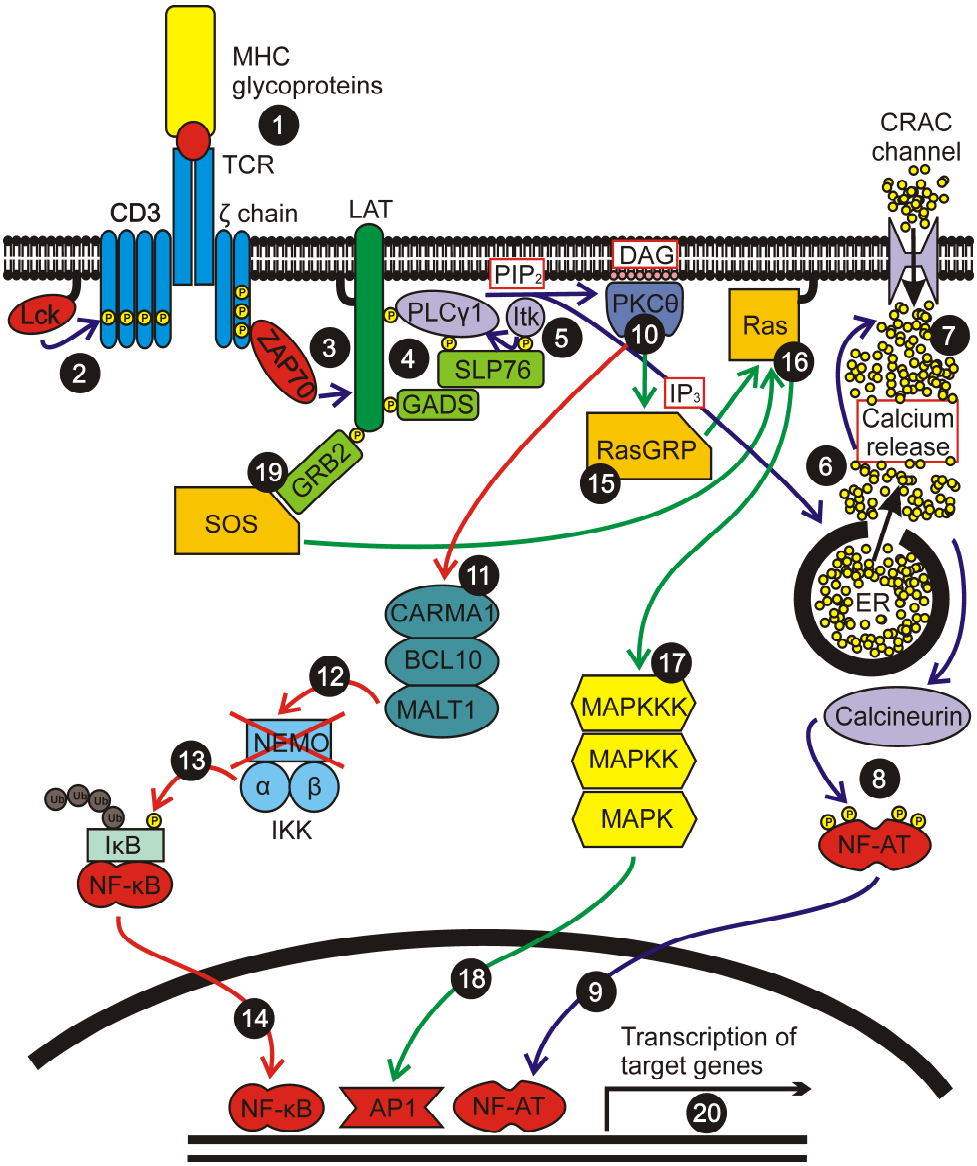

Figure 1. TCR induced signaling pathways.
Upon recognition of MHC glycoprotein loaded with specific peptide by TCR **(1)**, Src family kinase Lck is activated and phosphorylates (P) several immunoreceptor tyrosine-based activation motifs (ITAMs) on TCR associated CD3 subunits and ζ chains **(2)**. Zap70 binds to phosphorylated ITAMs and subsequently phosphorylates LAT **(3)**. LAT associates with GADS-SLP76-PLCγ1 complex **(4)** and PLCγ1 becomes activated by Itk and cleaves membrane lipid PIP_2 to cytoplasmic IP_3 and membrane bound DAG **(5)**. IP_3 promotes release of calcium from the endoplasmic reticulum (ER) **(6)** and this potentiates calcium flux from CRAC channels **(7)**. Increase in intracellular calcium activates phosphatase calcineurin that dephosphorylates transcription factor NF-AT **(8)** and this allows accumulation of NF-AT in the nucleus **(9)**. PLCγ1 produced DAG enables membrane recruitment and activation of PKCθ **(10)**, that initiates formation of CARMA1-BCL10-MALT1 complex **(11)** and cleavage of NEMO regulatory subunit of inhibitor of NF- κB (IκB) kinase (IKK) **(12)**. Active IKK phosphorylates IκB, that is subsequently polyubiquitinated and degraded **(13)**. Released transcription factor NF-κB then translocates into the nucleus **(14)**. PKCθ can also activate RasGRP **(15)** that activates membrane bound Ras **(16)** and this initiates the MAPK signaling pathway **(17)**, leading to activation of transcription factors such as AP-1 **(18)**. Ras can be activated also via membrane recruitment of GRB2-SOS to LAT **(19)**. Coordinated action of various transcription factors then regulates transcription of various genes **(20)**.

4.3 The signal propagation after MHCII crosslinking

The initiation and propagation of signaling from TCR has been studied in great detail. In contrast, precise knowledge of signaling pathways emanating from MHCII glycoproteins at IS is still lacking. Traditionally, MHCII glycoproteins were perceived as passives tools serving just for antigen presentation, not as receptors capable of signal transduction into the cells. However, a number of studies have provided the evidence that crosslinking of MHCII molecules using antibodies leads to various signaling events, including the activation of both Src and Syk family kinases, increase in overall tyrosine phosphorylation and calcium influx. Moreover, cytoskeleton rearrangement and cell aggregation have been described (24, 25), as well as triggering of distal signaling pathways including activation of MAPKs, NF-AT or PKCδ and PKCθ (26-28). Altogether, these data provide strong evidence that MHCII glycoproteins can propagate various signaling events.

The functional outcome of MHCII crosslinking is highly dependent on the cell type and the activation status. In both dendritic cells and monocytes MHCII ligation may induce cell death as well as maturation and expression of co-stimulatory molecules (29, 30). Ligation of MHCII on B cells produces caspase independent apoptosis, but also upregulation of co-stimulatory molecule CD86 (31, 32). From the clinical point of view, humanized antibodies targeting MHCII molecules represent interesting tools for treatment of B cell lymphomas. First, they can directly kill these cells and they also synergise with widely used drug rituximab, an antibody against CD20 (33-35).

The molecular mechanism of signal initiation after MHCII crosslinking is still unknown. Since MHCII possesses only a very short cytoplasmic tail, it cannot transmit signals on its own and must be coupled to other signaling molecules. Only few proteins were implicated to fulfill this function, namely Igα and Igβ (36), CD19 and CD20 (37). However, all these molecules are expressed in B cells only. Proteins responsible for MHCII signal transduction in other APC are not known; it also is not known whether in B cells there are other molecules involved in this pathway. It should be noted that one new protein involved in the signal propagation downstream of MHCII termed SCIMP has been identified in this thesis. Negative regulation of MHCII signaling is also mysterious, since so far, only a single protein, MPYS, has been shown to be phosphorylated upon MHCII triggering and to bind inhibitory molecules SHP1 and SHIP (27).

One of the possibilities, how MHCII glycoproteins could be connected to downstream signaling is the localization of MHCII molecules in some type of membrane microdomains, such as lipid rafts or tetraspanin microdomains.

4.4 Membrane microdomains - tetraspanin enriched microdomains and lipid rafts.

Tetraspanins form a large protein family with 33 members described both in human and mouse. All tetraspanins are characterized by a conserved structure of four transmembrane domains separated by two extracellular loops (one small and one large loop of characteristic composition) and one intracellular loop. Both N- and C- terminal ends face the cytoplasm. The intracellular parts of tetraspanins are palmitoylated on several submembrane cysteine residues (38).

With very few exceptions, tetraspanins do not function as surface receptors. Instead, they interact with various membrane proteins, including ICAM-1, VCAM-1, integrins (38), CD19 (39), EWI-F, EWI-2 (40) and importantly also MHCII molecules (41, 42). Direct interactions between particular protein and a specific member of tetraspanin family are called ”first level interactions“. These associations are resistant to relatively strong detergents such as Triton-X100. ”Second level interactions“ are mediated by clustering of individual tetraspanins together. Since tetraspanins are bound to particular membrane proteins, this promotes an indirect cross-talk between various tetraspanin associated proteins. Indeed, tetraspanins are known to immunoprecipitate with each other after lysis in certain relatively mild detergents, such as CHAPS or Brij98 (43). Finally, ”third level interactions“ represent large aggregates of tetraspanins and tetraspanin associated proteins, as well as several intracellular signaling molecules, including PKCθ or phosphoinoside 4-kinase (38). Resulting membrane domains are also known as tetraspanin-enriched microdomains (TEMs) (44). The most likely function of TEMs is in the formation of membrane platforms, where different receptors, membrane molecules and signaling molecules might be enriched based on their preferences for association with particular tetraspanin. As a result, different receptors and effector proteins could be coupled together without the necessity of being direct interacting partners.

It should be noted that even though much of our knowledge of tetraspanin biology has been gained using biochemical methods and various detergents, the tetraspanin domains have been detected also in the living cells using TIRF microscopy (45).

Opposite to TEMs that are based on protein-protein interactions, lipid rafts are based on protein-lipid and lipid-lipid interactions. They are enriched in sphingolipids, cholesterol and saturated phospholipids and certain acylated or GPI linked proteins. In the resting state, lipid rafts are nanoscale and highly dynamic domains, that might coalesce in larger raft platforms upon stimulation (46).

The historically most often used method for lipid raft isolation is dependent on the extraction of cellular membranes with non-ionic detergents. However, since great number of detergents of varying concentrations and efficiencies were used by different research groups, a lot of controversy has arisen (47). As a standard, resistance of proteins to extraction using Triton X-100 or NP-40 detergents is usually accepted as a biochemical proof of their localization into “classical“ lipid rafts. It should be noted that these detergents are dissolving TEMs. Using milder detergents (such as Brij98 or CHAPS), both TEMs and lipid rafts can be isolated and it is not possible to distinguish whether proteins resisting extraction with these mild detergents are part of TEMs or lipid rafts. Therefore tetraspanins were described in several publications as constituents of lipid rafts, but current view is that TEMs and lipid rafts are two separate types of domains (48).

Analysis of protein resistance to extraction with particular detergents is a very convenient method for determining its association with lipid rafts, due to its relative simplicity. On the other hand, this method is prone to artifacts and more subtle methods based on high-resolution microscopy and lipidomics are now used for studying composition of lipid rafts (46).

4.5 Regulation of Src family kinases

Function of both TEMs and lipid rafts is to facilitate interaction among various proteins, such as transmembrane receptors and signal propagating molecules. Src family kinases, which are enriched mainly in lipid rafts (49), but were reported also in association with tetraspanin CD63 (50), play a prominent role in cell signaling, since they are the first signal transducing molecules to be activated upon triggering of various surface receptors.

Family of Src kinases comprises eight members: Src, Lck, Fyn, Lyn, Hck, Yes, Fgr, Blk and Yrk. All Src kinases share the same structure: acylation site at N-terminus is followed by unique domain (this domain differs the most among individual members of Src family), SH3 domain, SH2 domain, kinase domain and C-terminal regulatory tail (51). Regulation of the Src kinase activity is mediated by two critical tyrosines – inhibitory tyrosine located in the C-terminus (Y527 in Src) and activatory tyrosine located in the kinase domain (Y416 in Src).

Analysis of crystal structures revealed that intramolecular binding of SH2 domain to inhibitory tyrosine holds Src kinases in the inactive conformation, that is further stabilized by the interaction of the SH3 domain with the linker region between the SH2 domain and the kinase domain (51). Phosphorylation of the inhibitory tyrosine is mediated by C-terminal Src kinase (Csk). Opposing Csk are phosphatases CD45 and CD148 that can dephosphorylate this inhibitory tyrosine. However, for full activation, Src kinases must also auto-phosphorylate the activatory tyrosine in the kinase domain. This activatory tyrosine might be also substrate for CD45, and as demonstrated in this thesis also for CD148.

4.5.1 Mechanism of Src family kinases regulation by Csk

Csk is the main negative regulator of Src family kinase activity. Indeed, Csk knock-out mice die during embryonic development due to the defect in the neural tube development (52, 53), caused by aberrant activity of Src family kinases. Studies of mice with conditional Csk deletion in thymocytes have demonstrated that Csk-deficient T cells can evolve even without TCR (54) due to the strong signal obtained from deregulated kinases Lck and Fyn. In the triple knock-out of Lck, Fyn, and Csk this phenotype is reversed (55). Accordingly, Csk-deficient granulocytes are hyperresponsive to various types of stimulations (56). These data clearly demonstrate that Csk mediated regulation of Src family kinases is mandatory for proper coupling of surface receptors to intracellular signaling pathways.

Domain organization of Csk is similar to Src family kinases, since it contains an SH2 domain, an SH3 domain and a C-terminal kinase domain. However, in contrast to Src family kinases, Csk does not contain an acylation motif for membrane anchoring. Moreover, Csk possesses neither inhibitory nor activatory tyrosines present in Src. In order to exert its functions, Csk must bind via its SH2/SH3 module to the membrane anchored proteins. This positions Csk within the proximity of its substrates and it also facilitates conformational change in the Csk kinase domain from inactive to catalytically active state (57, 58). Several membrane anchored proteins have been described as Csk binding partners, including TRAPs termed phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG) (59) and Lck-interacting molecule (LIME) (60), or caveolin-1, or scaffolding protein paxilin (61).

Csk is also able to bind via its SH3 or SH2 domain to some members of PEST family of protein tyrosine phosphatases. These are cytoplasmic proteins capable of dephosphorylating Src kinases at their activatory tyrosines. A concomitant anchoring of Csk and PEST phosphatases to the membrane seems to serve as a strong negative regulator of cell activation (62).

4.5.2 Mechanism of Src kinases regulation by phosphatases CD45 and CD148

While the role of Csk is to phosphorylate inhibitory tyrosines of Src kinases, the main function of phosphatases CD45 and CD148 is to dephosphorylate this tyrosine and therefore release Src kinases from inactive state.

A comparison between CD45 and CD148 demonstrates that these proteins have rather different structures, even though they have very similar functions. CD45 contains a highly variable N-terminal part resulting from differential splicing depending on the cell type and maturation status. The variable part is followed by a conserved cysteine rich domain, three fibronectin type III repeats, a transmembrane domain, a submembrane wedge region regulating the phosphatase activity (63, 64), and two homologous phosphatase domains D1 and D2, of which only D1 is catalytically active (65). On the other hand, extracellular part of CD148 is not known to be subject to alternative splicing and consist of eight to nine fibronectin type III repeats. The transmembrane domain is followed again by the wedge domain and only one phosphatase domain (65).

Expression pattern is also different. While CD45 expression is restricted to nucleated hematopoietic cells, CD148 is broadly expressed including a number of non-immune tissues, with notable exception of mature naive murine T cells.

Mice deficient in CD45 have severe block in thymocyte development that is caused by defective signaling from pre-TCR and TCR due to the hyperphosphorylation of the inhibitory tyrosines of Src kinases (65-67). Accordingly, humans deficient in CD45 suffer from severe combined immmunodeficiency (SCID) (68-70). Suprisingly, analysis of B cells in CD45 deficient mice revealed that B cell numbers are relatively normal (66) and CD45-deficient B cells are able to signal via BCR, even though less efficiently than wild-type controls. However B cells, in contrast to mouse T cells, express CD148 phosphatase that can partially substitute for CD45 deficiency. Indeed, combined deficiency in CD45 and CD148 leads to a marked reduction of B cell numbers in the periphery and a highly defective signaling upon BCR activation (71). Thus, the weak phenotype of CD45 deficiency in the majority of hematopoietic cells outside the T cell compartment can be explained by redundant expression of CD148 (72).

Both CD45 and CD148 are known to be positive regulators of Src kinase activation. However, CD45 also functions as a negative regulator of cell activation, since activatory tyrosines of Src kinases are substrates for CD45. This phenomenon was nicely shown in the studies analyzing mice with various levels of CD45 expression (73, 74). Only 3% of

expression of CD45 compared to wild-type controls could rescue the T cells development. Increasing the CD45 expression up to 30% of the wild-type level further enhanced thymocyte responses, including calcium flux and Erk activation. However additional increase in CD45 expression resulted in downregulation of thymocyte responses. These data demonstrate that CD45 functions predominantly as positive regulator to a certain level of expression, while higher level of expression leads to increased dephosphorylation of activatory tyrosine as well. In can be concluded that CD45 (and also CD148, as demonstrated in this thesis) functions as a rheostat holding activity of Src kinases in the optimal range.

4.6 The role of adaptor proteins

Proper signal propagation is based on positioning of various proteins with enzymatic functions in order to enable their mutual interactions, access to the substrates and to the upstream regulators. Important molecules responsible for spatio-temporal organization of signal propagating molecules are adaptors proteins. They do not posses any enzymatic activity but organize and facilitate the activity of other signaling molecules. They can be divided into two groups depending on their localization - transmembrane or cytoplasmic adaptor proteins.

Transmembrane adaptors, or TRAPs, contain a short extracellular part, a transmembrane domain and cytoplasmic part with various protein-protein interacting motifs. Several TRAPs have a submembrane palmitoylation motif that positions them in the lipid rafts – namely non-T cell activation linker (NTAL), PAG, LIME and LAT. On the other hand, TRAPs without a palmitoylation motif have also been described – linker for activation of X cells (LAX), SH2-domain-containing protein tyrosine phosphatase (SHP2)-interacting TRAP (SIT) and TCR-interacting molecule (TRIM). The function of TRAPs is to anchor effector molecules at the plasma membrane upon stimulation of various receptors and thus enable the formation of larger signalosomes, such as in the case of LAT (75).

Similar to TRAPs, cytoplasmic adaptor proteins also serve as scaffolding proteins enabling interaction of various signaling molecules. For the purpose of this thesis, two important families of cytoplasmic adaptors involved in the immunoreceptor signaling are described in the next chapters.

4.6.1 SLP76 and SLP65

SLP76 and SLP65 (known also as BLNK or BASH) are two closely related proteins. SLP76 is expressed mainly in T cells, NK cells, mast cells, platelets, neutrophils, macrophages, dendritic cells and developing B cells. Notably it is absent in mature B cells. SLP65 is expressed mainly in B cells and macrophages (76).

SLP76 contains a C-terminal SH2 domain, a central proline rich domain binding both PLCγ1 and GADS and an N-terminal part with several tyrosines that enable interactions with various signaling molecules, as described below. In order to function, SLP76 must be anchored to the plasma membrane. In T cells, this is mediated by binding of the complex GADS-SLP76 to phosphorylated LAT. PLCγ1 then binds to both SLP76 and LAT. Subsequently SLP76 becomes phosphorylated by Syk family kinases and binds Itk, the activator of PLCγ1. Activation of PLCγ within this complex then results in the initiation of calcium flux and downstream signaling events, as discussed in the introduction. However, SLP76 has also other functions. Binding of proteins Vav and non-catalytic region of tyrosine kinase (Nck) to phosphorylated SLP76 leads to the remodeling of actin cytoskeleton, while binding of the SH2 domain of SLP76 to an adaptor protein termed adhesion- and degranulation-promoting adaptor (ADAP) is important for “inside-out“ signaling, leading to the activation of integrins (76).

Organization of SLP65 is similar, except that at its N-terminal part it contains conserved leucine zipper (LZ) motif. In order to function, SLP65 must be also anchored to the plasma membrane. In contrast to SLP76, this can be mediated by several means. First, LZ motif via an unknown mechanism enables SLP65 to interact with the membrane (77). Furthermore, SLP65 can bind via its SH2 domain to the phosphorylated tyrosine in Igα subunit of B cell receptor (78). Finally, interaction with phosphorylated, ITAM bound Syk via SH2 domain of SLP65 has been described as a means for anchoring SLP65 and also for regulation of both SLP65 and Syk functions (79). After cell activation, SLP65 becomes phosphorylated and binds SH2 domains of PLCγ2 and its activator Btk, as well as Vav and Nck (76). Interestingly, SLP65 exists in two isoforms, differing in splicing of exon 8 coding proline rich domain, responsible for binding of the adaptor protein GRB2 (80).

Altogether, recruitement of both SLP65 and SLP76 on the membrane and their phosphorylation is a key step in downstream signaling events. Mouse deficient in SLP76 have severe block in T cells development, due to the defective signaling from from pre-TCR (81), signal transduction in mast cells upon high affinity Fcε receptor crosslinking is diminished (82) and platelets are unable to signal from collagen receptors (83). Moreover, mice deficient

in SLP76 suffer from subcutaneous haemorrhage caused by the defective separation of lymphatic and blood vascular systems (84). Similary, mouse deficient in SLP65 have block in B cell development, due to the defective signaling from pre-BCR (85, 86).

4.6.2 GRB2

GRB2 is closely related to two other proteins – already mentioned GADS and GRB2-related adaptor protein (GRAP). GRB2 is a cytoplasmic adaptor composed of N-terminal SH3 domain, central SH2 domain and C-terminal SH3 domain. It has broad tissue distribution and its general importance is well documented by the fact that mice deficient in GRB2 die early during embryogenesis (87).

As mentioned in the introduction, GRB2 binds via its SH3 domain to SOS. After T cell activation, GRB2 can bind via its SH2 domain phosphorylated LAT, therefore anchoring SOS at the plasma membrane, where it gets activated (7). In this way, GRB2 is an important regulator of Ras/MAPK activation.

Further function of GRB2 relies on its ability to oligomerize surface molecules. SOS1 can bind two GRB2 adaptors that can subsequently be bound to several phosphorylated tyrosines on LAT, leading to the clustering of these molecules (88). Since several other GRB2 binding molecules have been described, it can be a more general means of promoting protein aggregation after the cell activation.

In mice with conditional deletion of GRB2 in T cells, both positive and negative thymic selection are impaired and activation of MAPKs p38 and JNK upon TCR is markedly diminished (89). Interestingly, conditional deletion of GRB2 in B cells leads to increased calcium response and MAPKs activation. This phenotype, very opposite of that in T cells, can be explained by the observation that GRB2 deficient cells have markedly reduced phosphorylation of the inhibitory molecule CD22 upon activation (90). After phosphorylation, CD22 binds inhibitory phosphatases SHP1 and SHP2 that dephosphorylate several activatory proteins including Igα/Igβ and SLP65 and downregulate the signaling events (91). Thus it seems that in B cells, the main function of GRB2 is to connect the BCR signalosome to the inhibitory molecules such as CD22 rather than to activatory molecules such as SOS.

5 Aims of the study

The aim of this thesis was to study propagation and regulation of signaling from MHCII and TCR.

The first project describes the discovery of new TRAP that we named SCIMP. We focused on general description of this protein, with the emphasis on the expression analysis, biochemical characterization and identification of associated partners. Finally we proposed a role for SCIMP in MHCII signaling.

The second project analyzes the role of another TRAP known as PRR7. This protein has been so far described only in the brain while in the cells of the immune system it has never been studied. We analyzed its expression it T lymphocytes and proposed its potential role in the regulation of T cell apoptosis and TCR mediated responses.

The third project deals with the regulation of TCR signaling by CD148 phosphatase and clarified several issues that had been unresolved for some time. We thoroughly analyzed the expression of CD148 at various stages of thymocyte maturation and we provided evidence that CD148 can function as both positive and negative regulator of TCR signaling.

6 Results and discussion

6.1 SCIMP: transmembrane adaptor protein involved in MHCII signaling

6.1.1 Identification of SCIMP

Csk is an important cytoplasmic regulatory molecule that must be anchored at the plasma membrane to exert its function. In order to find new proteins interacting with Csk, we employed ScanProsite tool at ExPASy Proteomics Server (http://www.expasy.org/tools/scanprosite/) (92) to detect unknown proteins with a transmembrane domain and a consensus binding site for SH2 domain of Csk in the cytoplasmic domain. This approach led to the discovery of a new protein termed C17orf87. The purpose of this research was to provide basic characterization and to investigate the function of this new protein.

C17orf87 is a typical member of the TRAPs family, since it contains a very short extracellular part, a transmembrane domain, followed by a palmitoylation motif and in its intracellular part it possesses four potential tyrosine based protein-protein interaction motifs, a proline rich domain, but no obvious enzymatically active domain.

Based on the known preferences of a number of SH2 domains to bind to particular amino acid sequences containing phosphotyrosine, it was possible to predict binding partners for three of these tyrosines. Namely Grb2, SLP65/SLP76 and Csk. Based on these interactions, that were later confirmed biochemically, we decided to name this protein SCIMP, an abbreviation for SLP65/SLP76 and Csk Interacting Membrane Protein.

6.1.2 SCIMP expression profile

Since no information concerning SCIMP was available, we first performed expression analysis. The expression profile of a protein may be an important hint for elucidating its function. A wide expression of a protein indicate a general role in the organism (such as in the case of already mentioned GRB2 or Csk), while the restricted expression pattern may suggest a highly specialized role. Examples from the immune system may include the subunits of the TCR or BCR complexes.

The analysis of SCIMP expression on both mRNA transcript and protein level revealed that SCIMP is restricted to the immune system tissues, most notably spleen and lymph nodes. Moreover, within the immune system, SCIMP appeared to be expressed only in professional

APC (1, 2), such as monocytes, dendritic cells and B cells, but not in T cells, platelets or granulocytes. Accordingly, the expression of SCIMP highly correlated with the expression of MHCII in these cells. Altogether these results indicated that SCIMP might have a role in the process of antigen presentation.

6.1.3 Both SCIMP and MHCII are members of tetraspanin-enriched microdomains

SCIMP contains a highly conserved palmitoylation motif in its submembrane part. Using an elegant approach termed acyl-biotinyl exchange reaction (93) we provided evidence that SCIMP is S-acylated (most likely palmitoylated) at one of its submembrane cysteines (94).

Acylation of transmembrane proteins is often required for their association with membrane microdomains, such as lipid rafts or TEMs. We employed several approaches to establish SCIMP as a constituent of TEMs. First, TEMs are known to be localized in the uropod of migrating cells (40). Indeed SCIMP could also be detected in the uropod of spontaneously polarized Ramos cells. Second, TEMs are localized in IS on both T cell side and APC side (95). SCIMP was also translocated into IS and co-localized there with several members of tetraspanin family. Third, TEMs are resistant to the lysis in mild detergents CHAPS and Brij98, but not to the lysis in the lipid raft preserving detergent NP-40 (43). Using flotation in sucrose density gradient and gel filtration on sepharose 4B we observed exactly the same behavior for both SCIMP and several teteraspanins. Finally, we were able to co-immunoprecipitate SCIMP with several tetraspanins after cell lysis in mild detergents.

These results are of great interest, since MHCII molecules are also constituents of TEMs (41, 42, 96). Indeed, upon solubilization in Brij98, co-immunoprecipitation of SCIMP with MHCII molecules could be observed. It should be noted that this interaction was markedly weaker than interaction of SCIMP with tetraspanins. The most plausible explanation is that both SCIMP and MHCII molecules are components of TEMs, but not direct binding partners.

6.1.4 SCIMP in MHCII pathway

Expression profile, localization in TEMs and translocation into IS all pointed towards a role of SCIMP in MHCII signaling. Indeed in B cells, SCIMP became phosphorylated after MHCII crosslinking, but not after crosslinking of BCR. Moreover, we were able to confirm by immunoprecipitation the binding of GRB2, SLP65 (or SLP76 in monocytic cell line THP1), and Csk to phosphorylated SCIMP and interestingly also binding of Src kinase Lyn via its SH3 domain to the proline rich sequence of SCIMP.

6.1.5 Signaling mediated by SCIMP

Concomitant binding of SCIMP to both SLP65 and to Csk is a very interesting feature since these proteins have fundamentally opposing functions. Csk, as noted above, is a major negative regulator of Src family kinases and cell activation. On the other hand, SLP65 is an adaptor necessary for signal propagation. To function properly, both these proteins must be anchored to the plasma membrane. This raises an interesting question, whether SCIMP is an activatory molecule that brings SLP65 to the membrane or an inhibitory molecule that anchors Csk to the membrane.

In order to study the signals directly emanating from SCIMP, we employed a very useful method for studying transmembrane adaptor proteins. We prepared chimeric protein consisting of an extracellular part of CD25 fused to human SCIMP without initiation methionine. Crosslinking of this construct on the surface of B cells with anti-CD25 antibodies allowed us to crosslink CD25-SCIMP chimera and observe signaling pathways initiated by SCIMP alone.

Crosslinking of SCIMP chimera led to a markedly strong cellular response, including activation of PLCγ isoforms, calcium flux and Erk1/2 activation. Importantly, all these responses were lost when SLP65 binding site in CD25-SCIMP chimera was mutated. This finding clearly shows that SCIMP binds SLP65 in order to propagate signaling and it is in accord with known functions of SLP65.

An obvious question then was, what is the role of Csk. When we mutated the Csk binding site on CD25-SCIMP chimera, all cellular responses after SCIMP chimera crosslinking were enhanced. This provided evidence that Csk binding to SCIMP is important for negative regulation of signals emanating from SCIMP. To our knowledge SCIMP is the first protein known to bind both activatory SLP65/SLP76 molecules and the inhibitory Csk molecule. The role of Csk in the negative feedback loop regulating a particular protein is interesting, because often membrane anchored Csk is regarded as general regulator of Src kinase activity, rather than a specific regulator with effects limited to a particular pathway.

The binding of activatory molecule together with Csk might be a more general phenomenon of signal regulation. Indeed, a proteomic study searching for binding partners of SH2 domain of Csk have revealed a large group of various proteins (97), possibly indicating that the specificity of the inhibitory effect exerted by Csk is strongly dependent on its binding partner.

6.1.6 SCIMP as a regulator of MHCII mediated signal propagation

Discovery that crosslinking of CD25-SCIMP chimera leads to various signaling responses in the cell was intriguing. This led us to analyze the role played by SCIMP in MHCII signal transduction. We demonstrated that in murine K46 B cell line SCIMP deficiency resulted in fifty percent decrease of sustained Erk1/2 activity 10 minutes after MHCII activation. Moreover this phenotype could be rescued by transfecting the cells with human wild-type SCIMP, but not with SCIMP with mutated SLP65 binding site. Conversely, mutation of the Csk binding site led to an increase of Erk1/2 activation.

Altogether, this work provided basic characterization of so far unknown protein and demonstrated its role in the propagation of the signals emanating from MHCII. Further direction of SCIMP research should focus on the process of antigen presentation in mice deficient in SCIMP (currently in preparation).

6.2 PRR7 is a transmembrane adaptor protein expressed in activated T cells and involved in the regulation of T cell receptor signaling and apoptosis

6.2.1 Expression of PRR7 in the immune system

Another TRAP analyzed in our laboratory was proline rich 7 (PRR7). Similar to SCIMP, PRR7 was found in human genome database as typical member of TRAP family. It contains a short N-terminal extracellular part, transmembrane domain, palmitoylation motif and in its cytoplasmic part it contains a WW domain binding sequence, several potential tyrosine based interaction motifs, predicted SH3 domain binding motifs and a PDZ domain binding motif at its C-terminus. The name of this protein is derived from the notion that PRR7 contains high proportion of proline residues (98).

PRR7 has been described as a constituent of post synaptic density (PSD) of rat forebrain. There, PRR7 constitutively associates with scaffolding protein PSD95 and with two subunit of N-methyl-D-aspartate receptor (NMDAR), NR1 and NR2B (98). These data suggest that PRR7 might have a role in organizing the signaling network at the PSD fraction of neural synapse. Since neural and immune synapse share many similar features (99), the goal of this

project was to find out whether PRR7 is present in the immune system and what might be its potential role there.

Even though PRR7 is expressed most strongly in the brain, its mRNA transcripts are detectable in the majority of human tissues, including immune system organs thymus and lymph nodes. Furthermore, expression of PRR7 at both transcript and protein level is increased in peripheral blood lymphocytes upon stimulation with CD3 and CD28 antibodies as well as upon treatment of these cells with other mitogens.

6.2.2 Apoptosis induced by PRR7 expression

In order to provide further functional characterization of PRR7, Jurkat T cells transfected with PRR7 were prepared. However, such transfection resulted in rapid apoptotic death of these cells. This observation was verified also in Jurkat cell line with inducible expression of PRR7.

Further analysis of the regions of PRR7 responsible for apoptosis induction detected a critical region between amino acids 159-171. It is interesting that this sequence is part of WW domain binding motif that might be responsible for the interaction with some so far unknown regulatory proteins. Homologous sequence was described in protein WW domain binding protein 1 (WBP-1), where it mediates the interaction with various WW domain containing proteins including members of NEDD4 ubiquitin ligase family (100).

6.2.3 Effect of PRR7 on TCR signaling pathways

Explanation of the effect of overexpression of PRR7 on Jurkat T cells might lay in the ability of PRR7 to pre-activate some signaling pathways. Indeed, inducible expression of PRR7 resulted in increased surface expression of CD69 in Jurkat T cells. Moreover, while Jurkat wild-type cells produce very low level of IL-2 upon PMA and ionomycin stimulation, PRR7 expression sensitized these cells to produce large amounts of IL-2 upon this stimulation. At the same time, induction of PRR7 expression led to a highly increased expression c-Jun, a subunit of AP-1 transcription factor, phosphorylated on activatory serine (101). The most plausible explanation is that PMA and ionomycine treatment led to an activation of NF-κB and NF-AT transcription factors that synergized with PRR7-upregulated c-Jun in enhancing the production of IL-2. In the case of basal expression of CD69 upon PRR7 expression, the increased AP-1 activity is also likely responsible.

Since PRR7 potentiated the activity of distal signaling pathways, it was surprising that all the proximal signaling events generated after TCR crosslinking were decreased. Indeed,

upregulation of PRR7 led by so far unknown mechanism to reduced expression of Lck and reduced basal phosphorylation of TCR ζ chain. Accordingly, upon TCR stimulation, cells inducibly expressing PRR7 had weaker calcium response, decreased overall tyrosine phosphorylation and decreased phosphorylation of several signaling molecules including ZAP-70, LAT, PLCγ1, and Erk compared to control cells.

It seems that PRR7 induces signaling events leading to c-Jun activation and subsequently to apoptosis. This is counterbalanced by decreased ability of the cells to signal from TCR, probably due to a so far unknown negative feedback or due to cell exhaustion and incapability to support further signaling events. The fact that PRR7 is expressed in activated lymphocytes points to a possible role of PRR7 in the regulation of T cell activation. Whether PRR7 really functions as a regulator of T cell activation also *in vivo* should be addressed in further research focusing on analysis of knock-out mice.

6.3 Regulation of Src family kinases involved in T cell receptor signaling by protein-tyrosine phosphatase CD148

6.3.1 Expression profile of phosphatase CD148

CD148 is an important regulator of Src kinase activity. However, several issues concerning this phosphatase had been unresolved. Early data concerning the expression of CD148 demonstrated the difference between mouse and human. While mouse naive mature T cells were reported as CD148 negative (72), human T cells were demonstrated to be positive for CD148 (102). However, no thorough analysis of the differences in CD148 expression was carried out. In the first part of this study the expression pattern of CD148 was analyzed in various developmental stages of human and mouse thymocytes.

Evidence was provided that in mice, double negative (DN) T cells express CD148. However, this expression is lost as T cells progress to single positive (SP) stage. Interestingly, human thymocytes exhibited different pattern, with non-detectable CD148 on DN thymocytes, while strong expression was observed on mature SP cells.

6.3.2 Phosphatase CD148 can dephosphorylate inhibitory tyrosine of Src family kinases in T cells

CD148 was shown to complement CD45 as a positive regulator of BCR and Fc receptor signaling (71). Whether CD148 might promote also TCR signaling in CD45 negative cells had not been previously addressed. In this work evidence was provided that in CD45 negative cell line JS-7 (with low basal level of CD148) a forced expression of CD148 led to an increase in overall tyrosine phosphorylation upon TCR stimulation accompanied by enhanced calcium response and CD69 upregulation. This was in accord with the observation that phosphorylation of inhibitory tyrosines of both Lck and Fyn was reduced. The direct link between CD148 and Lck and Fyn was provided by co-immunoprecipitation of CD148 with both kinases. Altogether, these data clearly demonstrated that CD148 can have the same positive effect on TCR signaling as CD45.

This notion is seemingly in conflict with previous observation showing that forced expression of CD148 in CD45 positive Jurkat T cells has negative effect on the phosphorylation of PLCγ1 and LAT upon TCR stimulation (103). However, there is an explanation for this discrepancy. Since Jurkat cells already express CD45, these cells have sufficient phosphatase activity for dephosphorylating the inhibitory tyrosines of both Lck and Fyn, necessary for optimal TCR activation. Increasing phosphatase activity further with overexpression of CD148 leads to the dephosphorylation of the activatory tyrosines and therefore to the reduction of the Src kinase activity. Indeed, these conclusions are supported by our observation that forced expression of CD148 in Jurkat cells led to decreased phosphorylation of both activatory and inhibitory tyrosines.

Finally we prepared substrate trapping mutant of CD148 that can bind via phosphatase domain to its target phosphotyrosine, but cannot dephosphorylate it. This mutant can be used to identify potential substrates. In this assay, CD148 trapped Lck. Moreover, this Lck was hyperphosphorylated at both activatory and inhibitory tyrosines, demonstrating that both tyrosines are substrates of CD148.

Altogether these data demonstrate that CD148 can be positive as well as negative regulator of Src family kinases in T cells. The net effect of CD148 is dependent on overall combined phosphatase activity of CD45 and CD148. Under the conditions when low phosphatase activity is present, Src would be hyperphosphorylated by Csk on the inhibitory tyrosine with low amount of active Src remaining available. If too much phosphatase acitivity is present, Src kinases would be hypophosphorylated on the activatory tyrosine, leading again to suboptimal Src activity.

Suprisingly, patients deficient in CD45 suffer from SCID and CD148 cannot substitute for CD45 deficiency (68-70). This is probably reflecting the fact that the block in the development of T lymphocytes is at DP stage, when the expression of CD148 in human thymocytes is still low.

7 Conclusions

All projects presented in this thesis are related to the regulation of signal propagation. We report here the characterization of two TRAPs in the immune system. The first of them is a new protein that we named SCIMP. We thoroughly analyzed this protein and provided evidence that SCIMP is expressed in APCs, it is translocated into the IS and propagate signaling emanating from MHCII molecules due to its binding to SLP65/SLP76. Interestingly, signaling events emanating from SCIMP are negatively regulated by concomitant binding of Csk. The second TRAP analyzed is PRR7, a protein that has been so far described only in the brain. We provided evidence that PRR7 is also expressed in T cells. Overexpression of PRR7 in Jurkat T cell line led to cell apoptosis and attenuated TCR signaling, probably due to the pre-activation and exhaustion of the cells and/or reduced expression of Src-family kinase Lck. We concluded that expression of PRR7 might be an interesting mechanism of the regulation of T cell functions.

Both SCIMP and PRR7 are able to regulate Src family kinases. While PRR7 downmodulates the expression level of Src-family member Lck, SCIMP presumably regulates these enzymes via recruiting their negative regulator Csk which phosphorylates their inhibitory tyrosines. Yet another regulatory mechanism is represented by CD148. We provided evidence that CD148 is capable of dephosphorylating this inhibitory tyrosine. On the other hand, it can also dephosphorylate activatory tyrosine of Src family kinases and thus function as both positive and negative regulator of immunoreceptor signaling. Furthermore we clarified unresolved issue of CD148 expression in thymus and demonstrated different expression of CD148 on human and mouse thymocytes at various developmental stages.

To summarize, we detected and provided basic characterization of two TRAPs involved in the regulation of APC (SCIMP) and T cell (PRR7) functions and we clarified the role of CD148 in signaling from TCR.

8 Contribution

1. **SCIMP: transmembrane adaptor protein involved in MHCII signaling.**
   In this study, I designed majority of published experiments, prepared vectors coding for various mutants of SCIMP or CD25-SCIMP chimeras and performed cell transfections, analyzed association of SCIMP with various proteins, performed microscopy analysis, prepared and analyzed cells expressing shRNA against SCIMP and participated on the writing of the manuscript.

2. **PRR7 is a transmembrane adaptor protein expressed in activated T cells involved in regulation of T cell receptor signaling and apoptosis.**
   In this project I tested anti-PRR7 antibodies prepared in our laboratory, performed basic biochemical analysis of this protein, and studied the expression of PRR7 in activated T cells.

3. **Regulation of Src family kinases involved in T cell receptor signaling by protein-tyrosine phosphatase CD148.**
   In this project I analyzed the association of CD148 with Src family kinases Lck and Fyn.

9 References

1. Savina A, Amigorena S. 2007. Phagocytosis and antigen presentation in dendritic cells. *Immunol Rev* 219: 143-56
2. Rodriguez-Pinto D. 2005. B cells as antigen presenting cells. *Cell Immunol* 238: 67-75
3. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. 1998. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 395: 82-6
4. Fooksman DR, Vardhana S, Vasiliver-Shamis G, Liese J, Blair DA, Waite J, Sacristan C, Victora GD, Zanin-Zhorov A, Dustin ML. 2010. Functional anatomy of T cell activation and synapse formation. *Annu Rev Immunol* 28: 79-105
5. Deindl S, Kadlecek TA, Brdicka T, Cao X, Weiss A, Kuriyan J. 2007. Structural basis for the inhibition of tyrosine kinase activity of ZAP-70. *Cell* 129: 735-46
6. Au-Yeung BB, Deindl S, Hsu LY, Palacios EH, Levin SE, Kuriyan J, Weiss A. 2009. The structure, regulation, and function of ZAP-70. *Immunol Rev* 228: 41-57
7. Balagopalan L, Coussens NP, Sherman E, Samelson LE, Sommers CL. 2010. The LAT story: a tale of cooperativity, coordination, and choreography. *Cold Spring Harb Perspect Biol* 2: a005512
8. Horejsi V, Otahal P, Brdicka T. 2010. LAT--an important raft-associated transmembrane adaptor protein. Delivered on 6 July 2009 at the 34th FEBS Congress in Prague, Czech Republic. *FEBS J* 277: 4383-97
9. Fuller DM, Zhang W. 2009. Regulation of lymphocyte development and activation by the LAT family of adapter proteins. *Immunol Rev* 232: 72-83
10. Oh-hora M, Rao A. 2008. Calcium signaling in lymphocytes. *Curr Opin Immunol* 20: 250-8
11. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA. 2005. STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J Cell Biol* 169: 435-45
12. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Stauderman KA, Cahalan MD. 2005. STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. *Nature* 437: 902-5
13. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. 2006. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441: 179-85
14. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. 2006. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 443: 230-3
15. Gwack Y, Feske S, Srikanth S, Hogan PG, Rao A. 2007. Signalling to transcription: store-operated Ca2+ entry and NFAT activation in lymphocytes. *Cell Calcium* 42: 145-56
16. Hayashi K, Altman A. 2007. Protein kinase C theta (PKCtheta): a key player in T cell life and death. *Pharmacol Res* 55: 537-44
17. Schulze-Luehrmann J, Ghosh S. 2006. Antigen-receptor signaling to nuclear factor kappa B. *Immunity* 25: 701-15
18. Roose JP, Mollenauer M, Gupta VA, Stone J, Weiss A. 2005. A diacylglycerol-protein kinase C-RasGRP1 pathway directs Ras activation upon antigen receptor stimulation of T cells. *Mol Cell Biol* 25: 4426-41
19. Dower NA, Stang SL, Bottorff DA, Ebinu JO, Dickie P, Ostergaard HL, Stone JC. 2000. RasGRP is essential for mouse thymocyte differentiation and TCR signaling. *Nat Immunol* 1: 317-21

20. Ebinu JO, Stang SL, Teixeira C, Bottorff DA, Hooton J, Blumberg PM, Barry M, Bleakley RC, Ostergaard HL, Stone JC. 2000. RasGRP links T-cell receptor signaling to Ras. *Blood* 95: 3199-203
21. Geest CR, Coffer PJ. 2009. MAPK signaling pathways in the regulation of hematopoiesis. *J Leukoc Biol* 86: 237-50
22. Su B, Karin M. 1996. Mitogen-activated protein kinase cascades and regulation of gene expression. *Curr Opin Immunol* 8: 402-11
23. Buday L, Downward J. 2008. Many faces of Ras activation. *Biochim Biophys Acta* 1786: 178-87
24. Lehner M, Stockl J, Majdic O, Knapp W, Huttner K, Felzmann T, Holter W. 2003. MHC class II antigen signaling induces homotypic and heterotypic cluster formation of human mature monocyte derived dendritic cells in the absence of cell death. *Hum Immunol* 64: 762-70
25. Ivanov A, Beers SA, Walshe CA, Honeychurch J, Alduaij W, Cox KL, Potter KN, Murray S, Chan CH, Klymenko T, Erenpreisa J, Glennie MJ, Illidge TM, Cragg MS. 2009. Monoclonal antibodies directed to CD20 and HLA-DR can elicit homotypic adhesion followed by lysosome-mediated cell death in human lymphoma and leukemia cells. *J Clin Invest* 119: 2143-59
26. Haylett RS, Koch N, Rink L. 2009. MHC class II molecules activate NFAT and the ERK group of MAPK through distinct signaling pathways in B cells. *Eur J Immunol* 39: 1947-55
27. Jin L, Waterman PM, Jonscher KR, Short CM, Reisdorph NA, Cambier JC. 2008. MPYS, a novel membrane tetraspanner, is associated with major histocompatibility complex class II and mediates transduction of apoptotic signals. *Mol Cell Biol* 28: 5014-26
28. Al-Daccak R, Mooney N, Charron D. 2004. MHC class II signaling in antigen-presenting cells. *Curr Opin Immunol* 16: 108-13
29. Bertho N, Drenou B, Laupeze B, Berre CL, Amiot L, Grosset JM, Fardel O, Charron D, Mooney N, Fauchet R. 2000. HLA-DR-mediated apoptosis susceptibility discriminates differentiation stages of dendritic/monocytic APC. *J Immunol* 164: 2379-85
30. Lokshin AE, Kalinski P, Sassi RR, Mailliard RB, Muller-Berghaus J, Storkus WJ, Peng X, Marrangoni AM, Edwards RP, Gorelik E. 2002. Differential regulation of maturation and apoptosis of human monocyte-derived dendritic cells mediated by MHC class II. *Int Immunol* 14: 1027-37
31. Drenou B, Blancheteau V, Burgess DH, Fauchet R, Charron DJ, Mooney NA. 1999. A caspase-independent pathway of MHC class II antigen-mediated apoptosis of human B lymphocytes. *J Immunol* 163: 4115-24
32. Nashar TO, Drake JR. 2006. Dynamics of MHC class II-activating signals in murine resting B cells. *J Immunol* 176: 827-38
33. Nagy ZA, Hubner B, Lohning C, Rauchenberger R, Reiffert S, Thomassen-Wolf E, Zahn S, Leyer S, Schier EM, Zahradnik A, Brunner C, Lobenwein K, Rattel B, Stanglmaier M, Hallek M, Wing M, Anderson S, Dunn M, Kretzschmar T, Tesar M. 2002. Fully human, HLA-DR-specific monoclonal antibodies efficiently induce programmed death of malignant lymphoid cells. *Nat Med* 8: 801-7
34. Stein R, Qu Z, Chen S, Solis D, Hansen HJ, Goldenberg DM. 2006. Characterization of a humanized IgG4 anti-HLA-DR monoclonal antibody that lacks effector cell functions but retains direct antilymphoma activity and increases the potency of rituximab. *Blood* 108: 2736-44

35. Brown KS, Levitt DJ, Shannon M, Link BK. 2001. Phase II trial of Remitogen (humanized 1D10) monoclonal antibody targeting class II in patients with relapsed low-grade or follicular lymphoma. *Clin Lymphoma* 2: 188-90
36. Lang P, Stolpa JC, Freiberg BA, Crawford F, Kappler J, Kupfer A, Cambier JC. 2001. TCR-induced transmembrane signaling by peptide/MHC class II via associated Ig-alpha/beta dimers. *Science* 291: 1537-40
37. Leveille C, Castaigne JG, Charron D, Al-Daccak R. 2002. MHC class II isotype-specific signaling complex on human B cells. *Eur J Immunol* 32: 2282-91
38. Charrin S, le Naour F, Silvie O, Milhiet PE, Boucheix C, Rubinstein E. 2009. Lateral organization of membrane proteins: tetraspanins spin their web. *Biochem J* 420: 133-54
39. Shoham T, Rajapaksa R, Kuo CC, Haimovich J, Levy S. 2006. Building of the tetraspanin web: distinct structural domains of CD81 function in different cellular compartments. *Mol Cell Biol* 26: 1373-85
40. Sala-Valdes M, Ursa A, Charrin S, Rubinstein E, Hemler ME, Sanchez-Madrid F, Yanez-Mo M. 2006. EWI-2 and EWI-F link the tetraspanin web to the actin cytoskeleton through their direct association with ezrin-radixin-moesin proteins. *J Biol Chem* 281: 19665-75
41. Angelisova P, Hilgert I, Horejsi V. 1994. Association of four antigens of the tetraspans family (CD37, CD53, TAPA-1, and R2/C33) with MHC class II glycoproteins. *Immunogenetics* 39: 249-56
42. Rubinstein E, Le Naour F, Lagaudriere-Gesbert C, Billard M, Conjeaud H, Boucheix C. 1996. CD9, CD63, CD81, and CD82 are components of a surface tetraspan network connected to HLA-DR and VLA integrins. *Eur J Immunol* 26: 2657-65
43. Yanez-Mo M, Barreiro O, Gordon-Alonso M, Sala-Valdes M, Sanchez-Madrid F. 2009. Tetraspanin-enriched microdomains: a functional unit in cell plasma membranes. *Trends Cell Biol* 19: 434-46
44. Zoller M. 2009. Tetraspanins: push and pull in suppressing and promoting metastasis. *Nat Rev Cancer* 9: 40-55
45. Espenel C, Margeat E, Dosset P, Arduise C, Le Grimellec C, Royer CA, Boucheix C, Rubinstein E, Milhiet PE. 2008. Single-molecule analysis of CD9 dynamics and partitioning reveals multiple modes of interaction in the tetraspanin web. *J Cell Biol* 182: 765-76
46. Simons K, Gerl MJ. 2010. Revitalizing membrane rafts: new tools and insights. *Nat Rev Mol Cell Biol* 11: 688-99
47. Shaw AS. 2006. Lipid rafts: now you see them, now you don't. *Nat Immunol* 7: 1139-42
48. Hemler ME. 2005. Tetraspanin functions and associated microdomains. *Nat Rev Mol Cell Biol* 6: 801-11
49. Zajchowski LD, Robbins SM. 2002. Lipid rafts and little caves. Compartmentalized signalling in membrane microdomains. *Eur J Biochem* 269: 737-52
50. Skubitz KM, Campbell KD, Iida J, Skubitz AP. 1996. CD63 associates with tyrosine kinase activity and CD11/CD18, and transmits an activation signal in neutrophils. *J Immunol* 157: 3617-26
51. Bradshaw JM. 2010. The Src, Syk, and Tec family kinases: distinct types of molecular switches. *Cell Signal* 22: 1175-84
52. Imamoto A, Soriano P. 1993. Disruption of the csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice. *Cell* 73: 1117-24

53. Nada S, Yagi T, Takeda H, Tokunaga T, Nakagawa H, Ikawa Y, Okada M, Aizawa S. 1993. Constitutive activation of Src family kinases in mouse embryos that lack Csk. *Cell* 73: 1125-35
54. Schmedt C, Saijo K, Niidome T, Kuhn R, Aizawa S, Tarakhovsky A. 1998. Csk controls antigen receptor-mediated development and selection of T-lineage cells. *Nature* 394: 901-4
55. Schmedt C, Tarakhovsky A. 2001. Autonomous maturation of alpha/beta T lineage cells in the absence of COOH-terminal Src kinase (Csk). *J Exp Med* 193: 815-26
56. Thomas RM, Schmedt C, Novelli M, Choi BK, Skok J, Tarakhovsky A, Roes J. 2004. C-terminal SRC kinase controls acute inflammation and granulocyte adhesion. *Immunity* 20: 181-91
57. Mikkola ET, Gahmberg CG. 2010. Hydrophobic interaction between the SH2 domain and the kinase domain is required for the activation of Csk. *J Mol Biol* 399: 618-27
58. Chong YP, Mulhern TD, Cheng HC. 2005. C-terminal Src kinase (CSK) and CSK-homologous kinase (CHK)--endogenous negative regulators of Src-family protein kinases. *Growth Factors* 23: 233-44
59. Brdicka T, Pavlistova D, Leo A, Bruyns E, Korinek V, Angelisova P, Scherer J, Shevchenko A, Hilgert I, Cerny J, Drbal K, Kuramitsu Y, Kornacker B, Horejsi V, Schraven B. 2000. Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. *J Exp Med* 191: 1591-604
60. Brdickova N, Brdicka T, Angelisova P, Horvath O, Spicka J, Hilgert I, Paces J, Simeoni L, Kliche S, Merten C, Schraven B, Horejsi V. 2003. LIME: a new membrane Raft-associated adaptor protein involved in CD4 and CD8 coreceptor signaling. *J Exp Med* 198: 1453-62
61. Cao H, Sanguinetti AR, Mastick CC. 2004. Oxidative stress activates both Src-kinases and their negative regulator Csk and induces phosphorylation of two targeting proteins for Csk: caveolin-1 and paxillin. *Exp Cell Res* 294: 159-71
62. Veillette A, Rhee I, Souza CM, Davidson D. 2009. PEST family phosphatases in immunity, autoimmunity, and autoinflammatory disorders. *Immunol Rev* 228: 312-24
63. Hermiston ML, Tan AL, Gupta VA, Majeti R, Weiss A. 2005. The juxtamembrane wedge negatively regulates CD45 function in B cells. *Immunity* 23: 635-47
64. Hermiston ML, Zikherman J, Tan AL, Lam VC, Cresalia NM, Oksenberg N, Goren N, Brassat D, Oksenberg JR, Weiss A. 2009. Differential impact of the CD45 juxtamembrane wedge on central and peripheral T cell receptor responses. *Proc Natl Acad Sci U S A* 106: 546-51
65. Hermiston ML, Zikherman J, Zhu JW. 2009. CD45, CD148, and Lyp/Pep: critical phosphatases regulating Src family kinase signaling networks in immune cells. *Immunol Rev* 228: 288-311
66. Byth KF, Conroy LA, Howlett S, Smith AJ, May J, Alexander DR, Holmes N. 1996. CD45-null transgenic mice reveal a positive regulatory role for CD45 in early thymocyte development, in the selection of CD4+CD8+ thymocytes, and B cell maturation. *J Exp Med* 183: 1707-18
67. Mee PJ, Turner M, Basson MA, Costello PS, Zamoyska R, Tybulewicz VL. 1999. Greatly reduced efficiency of both positive and negative selection of thymocytes in CD45 tyrosine phosphatase-deficient mice. *Eur J Immunol* 29: 2923-33
68. Kung C, Pingel JT, Heikinheimo M, Klemola T, Varkila K, Yoo LI, Vuopala K, Poyhonen M, Uhari M, Rogers M, Speck SH, Chatila T, Thomas ML. 2000. Mutations

in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease. *Nat Med* 6: 343-5
69. Tchilian EZ, Wallace DL, Wells RS, Flower DR, Morgan G, Beverley PC. 2001. A deletion in the gene encoding the CD45 antigen in a patient with SCID. *J Immunol* 166: 1308-13
70. Cale CM, Klein NJ, Novelli V, Veys P, Jones AM, Morgan G. 1997. Severe combined immunodeficiency with abnormalities in expression of the common leucocyte antigen, CD45. *Arch Dis Child* 76: 163-4
71. Zhu JW, Brdicka T, Katsumoto TR, Lin J, Weiss A. 2008. Structurally distinct phosphatases CD45 and CD148 both regulate B cell and macrophage immunoreceptor signaling. *Immunity* 28: 183-96
72. Lin J, Zhu JW, Baker JE, Weiss A. 2004. Regulated expression of the receptor-like tyrosine phosphatase CD148 on hemopoietic cells. *J Immunol* 173: 2324-30
73. McNeill L, Salmond RJ, Cooper JC, Carret CK, Cassady-Cain RL, Roche-Molina M, Tandon P, Holmes N, Alexander DR. 2007. The differential regulation of Lck kinase phosphorylation sites by CD45 is critical for T cell receptor signaling responses. *Immunity* 27: 425-37
74. Zikherman J, Jenne C, Watson S, Doan K, Raschke W, Goodnow CC, Weiss A. 2010. CD45-Csk phosphatase-kinase titration uncouples basal and inducible T cell receptor signaling during thymic development. *Immunity* 32: 342-54
75. Horejsi V, Zhang W, Schraven B. 2004. Transmembrane adaptor proteins: organizers of immunoreceptor signalling. *Nat Rev Immunol* 4: 603-16
76. Koretzky GA, Abtahian F, Silverman MA. 2006. SLP76 and SLP65: complex regulation of signalling in lymphocytes and beyond. *Nat Rev Immunol* 6: 67-78
77. Kohler F, Storch B, Kulathu Y, Herzog S, Kuppig S, Reth M, Jumaa H. 2005. A leucine zipper in the N terminus confers membrane association to SLP-65. *Nat Immunol* 6: 204-10
78. Engels N, Wollscheid B, Wienands J. 2001. Association of SLP-65/BLNK with the B cell antigen receptor through a non-ITAM tyrosine of Ig-alpha. *Eur J Immunol* 31: 2126-34
79. Kulathu Y, Hobeika E, Turchinovich G, Reth M. 2008. The kinase Syk as an adaptor controlling sustained calcium signalling and B-cell development. *EMBO J* 27: 1333-44
80. Grabbe A, Wienands J. 2006. Human SLP-65 isoforms contribute differently to activation and apoptosis of B lymphocytes. *Blood* 108: 3761-8
81. Clements JL, Yang B, Ross-Barta SE, Eliason SL, Hrstka RF, Williamson RA, Koretzky GA. 1998. Requirement for the leukocyte-specific adapter protein SLP-76 for normal T cell development. *Science* 281: 416-9
82. Pivniouk VI, Martin TR, Lu-Kuo JM, Katz HR, Oettgen HC, Geha RS. 1999. SLP-76 deficiency impairs signaling via the high-affinity IgE receptor in mast cells. *J Clin Invest* 103: 1737-43
83. Clements JL, Lee JR, Gross B, Yang B, Olson JD, Sandra A, Watson SP, Lentz SR, Koretzky GA. 1999. Fetal hemorrhage and platelet dysfunction in SLP-76-deficient mice. *J Clin Invest* 103: 19-25
84. Abtahian F, Guerriero A, Sebzda E, Lu MM, Zhou R, Mocsai A, Myers EE, Huang B, Jackson DG, Ferrari VA, Tybulewicz V, Lowell CA, Lepore JJ, Koretzky GA, Kahn ML. 2003. Regulation of blood and lymphatic vascular separation by signaling proteins SLP-76 and Syk. *Science* 299: 247-51

85. Jumaa H, Wollscheid B, Mitterer M, Wienands J, Reth M, Nielsen PJ. 1999. Abnormal development and function of B lymphocytes in mice deficient for the signaling adaptor protein SLP-65. *Immunity* 11: 547-54
86. Pappu R, Cheng AM, Li B, Gong Q, Chiu C, Griffin N, White M, Sleckman BP, Chan AC. 1999. Requirement for B cell linker protein (BLNK) in B cell development. *Science* 286: 1949-54
87. Cheng AM, Saxton TM, Sakai R, Kulkarni S, Mbamalu G, Vogel W, Tortorice CG, Cardiff RD, Cross JC, Muller WJ, Pawson T. 1998. Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. *Cell* 95: 793-803
88. Houtman JC, Yamaguchi H, Barda-Saad M, Braiman A, Bowden B, Appella E, Schuck P, Samelson LE. 2006. Oligomerization of signaling complexes by the multipoint binding of GRB2 to both LAT and SOS1. *Nat Struct Mol Biol* 13: 798-805
89. Jang IK, Zhang J, Chiang YJ, Kole HK, Cronshaw DG, Zou Y, Gu H. 2010. Grb2 functions at the top of the T-cell antigen receptor-induced tyrosine kinase cascade to control thymic selection. *Proc Natl Acad Sci U S A* 107: 10620-5
90. Jang IK, Cronshaw DG, Xie LK, Fang G, Zhang J, Oh H, Fu YX, Gu H, Zou Y. 2011. Growth-factor receptor-bound protein-2 (Grb2) signaling in B cells controls lymphoid follicle organization and germinal center reaction. *Proc Natl Acad Sci U S A* 108: 7926-31
91. Nitschke L. 2009. CD22 and Siglec-G: B-cell inhibitory receptors with distinct functions. *Immunol Rev* 230: 128-43
92. de Castro E, Sigrist CJ, Gattiker A, Bulliard V, Langendijk-Genevaux PS, Gasteiger E, Bairoch A, Hulo N. 2006. ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. *Nucleic Acids Res* 34: W362-5
93. Wan J, Roth AF, Bailey AO, Davis NG. 2007. Palmitoylated proteins: purification and identification. *Nat Protoc* 2: 1573-84
94. Nadolski MJ, Linder ME. 2007. Protein lipidation. *FEBS J* 274: 5202-10
95. Mittelbrunn M, Yanez-Mo M, Sancho D, Ursa A, Sanchez-Madrid F. 2002. Cutting edge: dynamic redistribution of tetraspanin CD81 at the central zone of the immune synapse in both T lymphocytes and APC. *J Immunol* 169: 6691-5
96. Engering A, Pieters J. 2001. Association of distinct tetraspanins with MHC class II molecules at different subcellular locations in human immature dendritic cells. *Int Immunol* 13: 127-34
97. Yang G, Li Q, Ren S, Lu X, Fang L, Zhou W, Zhang F, Xu F, Zhang Z, Zeng R, Lottspeich F, Chen Z. 2009. Proteomic, functional and motif-based analysis of C-terminal Src kinase-interacting proteins. *Proteomics* 9: 4944-61
98. Murata Y, Doi T, Taniguchi H, Fujiyoshi Y. 2005. Proteomic analysis revealed a novel synaptic proline-rich membrane protein (PRR7) associated with PSD-95 and NMDA receptor. *Biochem Biophys Res Commun* 327: 183-91
99. Dustin ML, Colman DR. 2002. Neural and immunological synaptic relations. *Science* 298: 785-9
100. Pirozzi G, McConnell SJ, Uveges AJ, Carter JM, Sparks AB, Kay BK, Fowlkes DM. 1997. Identification of novel human WW domain-containing proteins by cloning of ligand targets. *J Biol Chem* 272: 14611-6
101. Davis RJ. 2000. Signal transduction by the JNK group of MAP kinases. *Cell* 103: 239-52

102. Autschbach F, Palou E, Mechtersheimer G, Rohr C, Pirotto F, Gassler N, Otto HF, Schraven B, Gaya A. 1999. Expression of the membrane protein tyrosine phosphatase CD148 in human tissues. *Tissue Antigens* 54: 485-98
103. Baker JE, Majeti R, Tangye SG, Weiss A. 2001. Protein tyrosine phosphatase CD148-mediated inhibition of T-cell receptor signal transduction is associated with reduced LAT and phospholipase Cgamma1 phosphorylation. *Mol Cell Biol* 21: 2393-403

10 Reprints of publications

1. Draber P, Vonkova I, Stepanek O, Hrdinka M, Kucova M, Skopcova T, Otahal P, Angelisova P, Horejsi V, Yeung M, Weiss A, Brdicka T. 2011. **SCIMP: transmembrane adaptor protein involved in MHCII signaling.** *Moll Cell Biol* [published ahead of print on 19 September 2011, doi:10.1128/MCB.05817-11]

2. Hrdinka M, Draber P, Stepanek O, Ormsby T, Otahal P, Angelisova P, Brdicka T, Paces J, Horejsi V, Drbal K. 2011. **PRR7 is a transmembrane adaptor protein expressed in activated T cells involved in regulation of T cell receptor signaling and apoptosis.** *J Biol Chem* 286: 19617-29

3. Stepanek O, Kalina T, Draber P, Skopcova T, Svojgr K, Angelisova P, Horejsi V, Weiss A, Brdicka T. 2011. **Regulation of Src family kinases involved in T cell receptor signaling by protein-tyrosine phosphatase CD148.** *J Biol Chem* 286: 22101-12