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New insights into inhibitory signaling cascades in immune cells: identification and characterization of a new Siglec-10 splice variant, Siglec-10 Sv3, and mapping of SHP-1 interactions with Siglec-10 and ILT2.

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Inhibitory signaling cascades are a crucial component of the immune system. Immune cells must be inhibited when they encounter molecules of the organism itself or harmless environmental antigens. If the inhibitory signaling pathway fails in this situation, it may result in immune disorders. However, the inhibition of immune cells in the moment of confrontation with pathogens or tumor cells, may lead to the pathogen-specific disease or malignancy. In order to find new approaches to the therapy of these disorders, steps of the inhibitory signaling cascades in immune cells were characterized in this dissertation.

A new protein of the cascade was identified and exact intracellular interactions of the signaling pathway were determined, using the three hybrid system strategy in yeast. This strategy is able to detect protein-protein interactions based on phosphorylation.

We focused on the interactions between the Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) and immune inhibitory receptors. Upon ligand binding these inhibitory receptors recruit SHP-1, which dephosphorylates further signaling molecules, finally resulting in cell inhibition. The structural basis for this recruitment consists of phosphorylated immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the cytoplasmic region of the inhibitory receptor and the SH2 domains in SHP-1. Our objectives were to map the exact docking sites of these interactions and to identify new proteins which recruit SHP-1.

1. We mapped the interaction between SHP-1 and the inhibitory receptor Ig-like transcript 2 (ILT2), a type I transmembrane protein expressed by different leukocyte lineages. Single tyrosine mutants of the intracellular ITIMs were generated, as well as single arginine mutants of the SH2 domains of SHP-1. β-galactosidase assays revealed ITIM Y614 as the main docking site for SHP-1 and ITIM Y644 as the co-operating docking site. According to other experiments it was expected that only one SH2 domain of SHP-1 was necessary for the interaction with ILT2. But surprisingly the interaction could only take place when both SH2 domains of SHP-1 were functional.

2. To identify novel inhibitory receptors to which SHP-1 might bind in a phosphotyrosine dependent manner, a PHA-activated PBMC library was screened in a three-hybrid system using SHP-1 as bait protein. Most positive clones turned out to be proteins known to interact with SHP-1, such as CD85 inhibitory receptors and LAIRs. But comparison to human cDNA databases revealed one unknown sequence with homologies to receptors of the Siglec-family.

After cloning the full length sequence, it was identified as a new splice variant of Siglec-10, which we called Siglec-10 Sv3. Siglec-10 Sv3 is located within the leukocyte receptor complex on chromosome 19q13.41 and belongs to the CD33 subgroup of Siglecs, since the alignment showed greatest homologies in sequence to CD33 (Siglec-3), Siglec-6 and Siglec-8. The original form, Siglec-10, is a type I transmembrane protein with one Ig-V and four Ig-C2 extracellular domains and a cytosolic tail with two ITIM motifs and one CD150-like (ITSM-like) sequence. The cytoplasmic tail is identical in Siglec-10 and its splice variants, but they differ in their extracellular regions. Compared to the 11 exons of Siglec-10, splice variant Siglec-10 Sv3 lacks the first 174 nucleotides of exon 3, which encode for the first 58 amino acids of the N-terminal Ig-C2 domain. Therefore, the first Ig-C2 domain of Siglec-10 Sv3 is probably not functional.

3. We characterized Siglec-10 Sv3 in terms of its expression, molecular weight and interaction with SHP-1. Using RT-PCR, Siglec-10 Sv3 was detected in PHA-activated peripheral blood lymphocytes. Its molecular weight of 90 kDa was determined by western blotting and immunoprecipitation. The interaction between Siglec-10 Sv3 and SHP-1 suggested by the 3HS screening, was reconfirmed by a 3HS assay as well as by western blotting and immunoprecipitation. In both experiments, the interactions were dependent on the presence of the Src kinase c-fyn mutant, indicating that Siglec-10 Sv3 tyrosine residues need to be phosphorylated to recruit SHP-1.

4. The recruitment of SHP-1 to the cytoplasmic tail of Siglec-10 was mapped using the 3HS. Mutation of the cytoplasmic tyrosine residues of Siglec-10 and the SH2 domains of SHP-1 indicates that ITIM Y609 recruits the N-terminal SH2 domain of SHP-1. In contrast to the SHP-1 recruitment to ILT2, it seems that Siglec-10 only needs one SH2 domain of SHP-1. Therefore, it can be hypothesized that the mechanism for SHP-1 recruitment depends on the recruiting molecule.

We can conclude that we have determined the exact docking sites for the interaction between SHP-1 and ILT-2, as well as for the interaction between SHP-1 and Siglec-10. Moreover we have identified a new splice variant of Siglec-10, Siglec-10 Sv3. These new insights into inhibitory processes contribute to a better understanding of inhibitory signaling, which we need in order to find new targets for therapy of cancer and immune disorders, such as autoimmune disease and allergy.