Summary

Resting leukocytes circulate throughout the body with the blood but have to leave the vessels and migrate into tissues to exert their immunological function. This extravasation during inflammatory responses or lymphocyte homing is mediated by the orchestrated interaction of several adhesion molecule families that constitute an adhesion cascade, resulting in firm adhesion of the leukocyte on the vessel wall and transmigration through the endothelial cells toward sites of inflammation or the lymph node. L-selectin, a carbohydrate-binding leukocytic adhesion molecule, mediates the first step in the cascade by transient interactions with vascular mucin-like glycoproteins, leading to capture of blood cells from the bloodstream and slow rolling along the vessel wall.

Additional to promoting cell-cell interactions, L-selectin also acts as a signalling molecule, activating several signal transduction cascades in the leukocyte. Moreover, L-selectin function is regulated by signals transmitted inside the cell (inside-out-signalling). An increase in binding activity is observed after treatment with chemokines and crosslinking of the T cell receptor complex and is thought to be induced by phosphorylation of L-selectin on serine residues in the cytoplasmic tail. Although many cellular effects of L-selectin-associated signalling have been described, the events occuring on the level of the receptor itself are little understood. This study therefore aimed at the identification of intracellular interaction partners of L-selectin that may be involved in signal generation by the receptor. A further object of this work was the characterisation of kinases responsible for phosphorylation of the receptor.

To achive this, the ability of serine protein kinases with basic recognition motifs to phosphorylate the cytoplasmic domain of L-selectin was tested. Both Protein Kinase C (PKC) and cGMP-dependent Kinase (PKG) could use a recombinant protein containing the intracellular sequence of the receptor as a substrate, whereas protein kinase A displayed no activity towards this protein.

By affinity isolation, a kinase activity was purified from cell lysates. This kinase bound tightly to the cytoplamic domain of L-selectin. and was able to phosphorylate this sequence on serine residues. Inhibition studies of this kinase showed that it was strongly inhibited by a specific PKC inhibitor. Western blotting experiments revealed the presence of two PKC isozymes associating with a Lsyto fusion protein: the novel PKCtheta and the atypical PKClambda.

The search for interaction partners that specifically bind to the serine-phosphorylated form of L-selectin yielded two proteins that were identified by mass spectrometry. These proteins proved to be PKC isozymes, conventional PKCα and PKCθ, which associated with phosphorylated L-selectin to a much larger extent than with the unmodified protein. Both PKCα and PKCθ co- immunoprecipitated with L-selectinfrom T-cell stimulated with phorbol ester, which has been shown previously to induce serine-phosphorylation of the receptor, proving that this interaction takes place in intact cells.

This study therefore shows, that PKC tightly associates with serine-phosphorylated L-selectin in an isozyme-specific manner and is likely to be also responsible for the phosphorylation of the receptor.