6 Summary

Together with its endothelial ligands, L-selectin mediates leukocyte rolling on vascular endothelium, thereby initiating the first step of an adhesion cascade that leads to transmigration of leukocytes into the perivascular tissue. On the one hand, L-selectin is thus essential for physiological immune defense; on the other hand, it is involved in pathological inflammatory reactions, e.g. transplant rejection or autoimmune diseases. It is therefore a target structure for antiinflammatory agents that inhibit leukocyte recruitment into inflamed tissue by L-selectin blockade.

The aim of this study was to examine the inhibition of L-selectin binding to its ligands by synthetic multivalent ligands on the basis of Sialyl Lewis x, glycomimetics and recombinant glycoproteins. These examinations were performed on the level of individual molecules and that of intact cells under flow conditions in order to take into account the shear stress dependence of L-selectin binding.

Different inhibitors were examined by developing a standardized test system that measured the binding of recombinant divalent L-selectin-IgG to the synthetic ligand $SiaLe^x$-PAA-sTyr by means of surface plasmon resonance. In addition, inhibitors were examined by adapting a flow chamber assay to measure the rolling and adhesion of transfected L-selectin-expressing NALM-6 cells on activated HUVECs.

Both procedures were used to measure the inhibitory activity of multivalent forms of Sialyl Lewis x, Sialyl Lewis a and tyrosine sulfate – covalently bound to the carrier polyacrylamide – in comparison to the monovalent forms of these ligands. The examinations showed that multivalence markedly enhances the inhibitory activity, and increasing shear stress intensifies the inhibitory effect of multivalent inhibitors.

Polyglycerol sulfate dendrimers were used to examine a second class of multivalent inhibitors. The results showed a remarkably strong inhibitory effect of these multivalent inhibitors. Polyglycerol sulfate dendrimers were specific for L- and P-selectin but did not inhibit the function of E-selectin.

Third, the macrolide efomycine M was used to examine the inhibitory activity of a glycomimetic. Efomycine M was specific for L-selectin but did not inhibit E- or P-selectin binding. These examinations showed that even monovalent inhibitors exert a strong inhibitory effect.

Two potential inhibitory glycoproteins were constructed as fusion proteins on the basis of the physiological ligand PSGL-1 and Hsc70, for which an L-selectin-binding cell surface form was recently described: PSGL-1-IgG as the fusion protein of the

N-terminus of PSGL-1 with three tyrosine residues and one *O*-glycan, which represent the L-selectin binding motive of PSGL-1, and Hsc70-IgG as the fusion protein of a sequence segment of Hsc70 of the position 279-299, which shows similarity to the PSGL-1 binding motive. The expression took place in the human cell line KG1a, which is suitable for glycosylation and sulfatation. Examinations utilizing surface plasmon resonance showed a comparably strong binding of PSGL-1-IgG and Hsc70-IgG to L-selectin. This result indicated that the recombinant glycoproteins PSGL-1-IgG and Hsc70-IgG are potent inhibitors of L-selectin function, at least in their divalent form.

The results of this study document the availability of multi- and monovalent inhibitors of L-selectin binding that open up prospects for the development of new anti-inflammatory drug strategies.