The Physiology and Pathophysiology of P-selectin

Markus Sperandio[1] and Klaus Ley[2]

[1]Children's Hospital, University of Heidelberg, Germany and [2]Cardiovascular Research Center, University of Virginia, Charlottesville, USA.

The multistep paradigm of leukocyte recruitment

The recruitment of leukocytes from the intravascular compartment into sites of inflamed tissue helps to protect vetrebrates from invading microorganims and other insults. Leukocyte recruitment follows a tightly regulated multistep adhesion cascade (Figure 1) starting with leukocyte capture to and rolling of free flowing leukocytes along the endothelial surface layer. This is followed by firm leukocyte adhesion to the endothelium and eventual transmigration through the endothelial layer (1,2).

Markus Sperandio, M.D., graduated from Freie Universität Berlin, School of Medicine, in 1995 and completed his doctoral thesis in physiology (Professor Dr. Peter Gaehtgens and Professor Dr. Axel Pries). Since 1996, he works at the Children's Hospital Heidelberg. From 1999 to 2002, he spent a postdoctoral fellowship in the laboratory of Professor Dr. Klaus Ley at the University of Virginia, Charlottesville, USA. **markus.sperandio@med.uni-heidelberg.de**

Leukocyte capture and rolling require the selectin family of adhesion molecules interacting with their counter-receptors called selectin ligands (3). Three different selectins have been identified, P-selectin on platelets and endothelial cells, E-selectin on endothelial cells, and L-selectin on most leukocytes. This review will focus on the physiology and pathophysiology of P-selectin considered to be a central player in mediating the first contact with and subsequent rolling of leukocytes along the inflamed endothelium.

Structure and genomic organization of P-selectin

P-selectin, formerly known as GMP-140 or PADGEM, belongs to a group of three mammalian lectins called selectins (4). Similar to the two other selectins, L- and E-selectin, P-selectin is a type I transmembrane glycoprotein (Figure 2) sharing a 50% sequence homology of its extracellular domain with the extracellular domain of E- and L-selectin. The gene for P-selectin was identified in a gene cluster on human chromosome 1 which contains also the genes for E- and L-selectin (5,6).

As illustrated in Figure 2, P-selectin contains a C-type lectin domain at the N-terminus (7) which recognizes crucial carbohydrate structures such as sialyl Lewis^x presented on selectin ligands. This was confirmed by a report on the crystal structure of P- and E-selectin bound to the tetrasaccharide sialyl Lewis^x (sLe^x) or the N-terminus of P-selectin glycoprotein ligand-1 (PSGL-1) (8). The EGF (epidermal growth factor)-like domain is not directly involved in binding of P-selectin to sLe^x (8). However, deletion of this domain dramatically reduces or abolishes adhesion of P-selectin (9,10). In humans, the EGF-like domain is followed by nine (eight in mice) consensus repeats (SCR) with homology to complement regulatory protein. Although P-selectin binding to its main ligand PSGL-1 does not require the SCRs (11), P-selectin expressing cells do not roll on PSGL-1 unless P-selectin is significantly spaced from the cell membrane (12). The cytoplasmic domain at the C-terminus of P-selectin and the transmembrane domain are required for sorting P-selectin into secretory granules (see below) (13,14).

Klaus Ley, M.D., received his medical degree from Julius-Maximilians Universität, Würzburg, where he also completed his doctoral thesis in physiology (Professor Dr. Hermann Henrich). After postdoctoral training in Berlin and at UCSD he accepted a junior faculty position (C1) at Freie Universität Berlin. In 1994, Ley moved to the University of Virginia, Charlottesville, where he is Professor of Biomedical Engineering, Molecular Physiology and BiologicalPhysics and became director of the Cardiovascular Research Center in 2001. **klausley@virginia.edu**

Function of P-selectin

P-selectin is stored in α-granules of platelets (15,16) and Weibel-Palade bodies of endothelial cells (17,18). Within seconds to minutes after stimulation, P-selectin is mobilized from its intracellular storage site to the surface membrane (18). This mobilization to the cell surface can be induced by such different molecules as TNF-α, LPS, thrombin, ADP, histamine, complement C5a and calcium ionophores (19). Besides the rapid route of mobilization from its storage pool, P-selectin expression can also be regulated via transcriptional upregulation involving NF-κB-activation. Interestingly, the transcriptional upregulation seems to be present only in mice, but not in humans (20,21). Surface-expressed P-selectin can be re-internalized and directed either to lysosomal granules (22) and to the trans-Golgi network, where it is resorted into newly formed Weibel-Palade bodies (23). Besides the surface bound P-selectin a shorter, soluble form of P-selectin exists resulting from an alternative splice variant excluding the transmembrane domain (24,25). The functional role of this variant is unknown but soluble P-selectin has been shown to induce the formation of microparticles from monocytes and neutrophils (26). Endothelial P-selectin functions as leukocyte rolling receptor and has a predominant role in leukocyte recruitment to sites of inflammation (27,28). This was demonstrated in P-selectin deficient mice, which showed reduced leukocyte recruitment into inflamed peritoneum

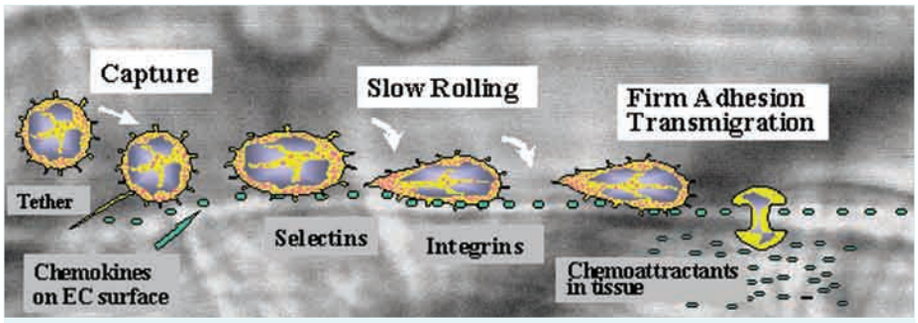

Figure 1: Multistep adhesion cascade of leukocyte recruitment. Leukocytes are captured from the blood stream by selectins, most commonly at the beginning of postcapillary venules followed by selectin-mediated rolling along the endothelium. Both capture and rolling involve the formation of leukocyte tethers, which can reach considerable lengths. During rolling, endothelial surface-bound chemokines act in concert with integrins to activate rolling leukocytes. Progressive activation leads to slowing down of the rolling leukocyte and eventually to firm adhesion and transmigration. Reproduced from (65). With Kind permission 57 Spinger Science and Business Media.

two hours after intraperitoneal injection of thioglycollate (29). In addition, leukocyte rolling normally observed immediately after the surgical preparation in cremaster muscle venules of wild type mice was not present in cremaster muscle venules of P-selectin deficient mice indicating that leukocyte rolling in this model is solely dependent on P-selectin solely dependent on P-selectin immediately after exteriorization (30). In contrast, in mice pretreated with the proinflammatory cytokine TNF-α, both P- and E-selectin are expressed (31) illustrating that P-selectin exerts its function as rolling receptor both in a distinct and in an overlapping fashion.

P-selectin ligands

P-selectin mediates leukocyte rolling via binding to P-selectin ligands. As already described above, binding involves the C-type lectin domain on P-selectin and crucial carbohydrate structures (core2 GlcNAcT-dependent O-glycans) on selectin ligands (32,3). Studies in mice deficient in glycosyltransferases such as fucosyltransferase-VII or core2 N-acetylglucosaminyl transferase-I (core 2 GlcNAcT-I) involved in the synthesis of core2 modified O-glycans on selectin ligands revealed the importance of posttranslational glycosylation on selectin ligand function (33-35). P-selectin glycoprotein-1 (PSGL-1), the main P-selectin ligand *in vivo*, is a type 1 transmembrane protein expressed as homodimer on microvilli of virtually all leukocytes (36). Mice deficient in PSGL-1 demonstrate a severe reduction in leukocyte rolling in untreated and TNF-α pretreated venules of the cremaster muscle (37). This reduction is similar to that seen in P-selectin deficient mice suggesting that PSGL-1 is the major ligand for P-selectin *in vivo* (37). CD24 (or heat stable antigen), a glycosyl-phosphatidyl (GPI)-anchored highly glycosylated cell surface glycoprotein expressed by neutrophils, B-lymphocytes, immature thymocytes, red blood cells and many tumor cells (3), mediates rolling of PSGL-1 negative tumor cell lines on P-selectin and suggests an important role of CD24 in metastasis of CD24 expressing tumor cells (38,39). However, a role of CD24 in leukocyte rolling has not been demonstrated.

P-selectin in human disease

There is a large body of evidence linking P-selectin to the pathogenesis of several human acute and chronic inflammatory conditions. Most prominently, P-selectin has been critically implicated in mediating leukocyte recruitment and subsequent tissue damage in many organs (f.e. heart, kidney, brain) exposed to ischemia/reperfusion (I/R) (40,41,42). In a feline model of myocardial ischemia and reperfusion injury, injection of the P-selectin blocking mAb PB1.3 eighty minutes after occlusion of the left anterior descending (LAD) coronary artery led to an almost 60% reduction in myocardial necrosis when assessed after 270 min reperfusion (43). In a murine model of myocardial I/R, endothelial P-selectin expression was upregulated in ischemic areas compared to non-ischemic areas (44) suggesting a role of P-selectin in mediating PMN accumulation into ischemic tissue. Indeed, P-selectin deficient mice when compared with control mice. This was in part due to the reduction of neutrophil infiltration into the ischemic kidney of P-selectin deficient mice (44). Interestingly, P-selectin-dependent leukocyte infiltration into the inner and outer medulla, being critical for the development of renal failure, was exclusively mediated by platelet expressed P-selectin but independent of endothelial P-selectin (45). Similar to myocardial and renal I/R models, cerebral ischemia-reperfusion

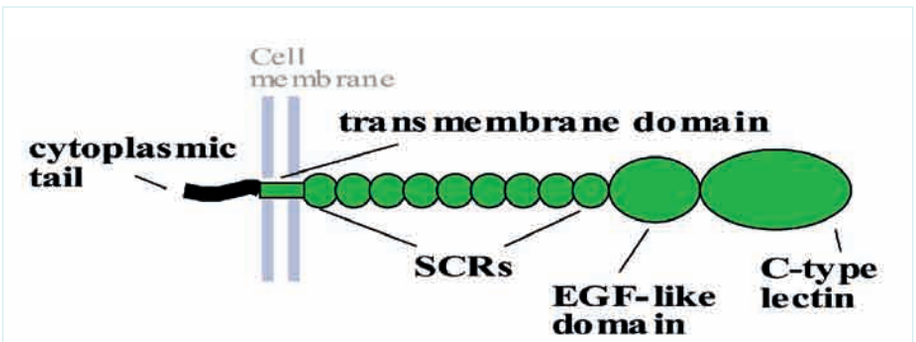

Figure 2: Structure of human P-selectin. The C-type lectin domain at the N-terminus binds to carbohydrate structures on P-selectin ligands in a Ca^{++}-dependent fashion. EGF-like domain and nine (eight in mice) consensus repeats following the lectin domain are not directly involved in ligand binding but indirectly influence the ability of P-selectin to recognize its ligands. The short cytoplasmic tail has sorting and signal transduction capabilities.

models in mice and rats demonstrated an upregulation of endothelial P-selectin in ischemic areas shortly after onset of cerebral ischemia (46,47). Furthermore, in P-selectin deficient mice or in mice pretreated with a blocking mAb against P-selectin, cerebral infarct size and postischemic survival was significantly improved compared to control mice (46). Similar results were found in rats treated with P-selectin blocking mAbs (48-50). In contrast, mice deficient in P- and E-selectin subjected to cerebral ischemia/reperfusion injury showed no significant difference in infarct size three hours and 21 hours after onset of ischemia (51) indicating that selectin-independent mechanisms contribute to ischemia/reperfusion injury after prolonged focal cerebral ischemia. In humans, no clinical trials have been conducted so far investigating the effects of P-selectin blockade in patients suffering from stroke, myocardial or renal infarction. However, a study in a primate hemispheric stroke model where baboons were subjected to treatment with a humanized monoclonal antibody against P- and E-selectin (HuEP5C7) immediately after onset of ischemia showed a beneficial outcome with a decrease in infarct volume and a better neurological score in HuEP5C7 treated animals (52). These results may stimulate clinical trials in stroke patients with the intention to block P-selectin immediately after a stroke had occurred.

In patients with atherosclerosis, P-selectin was shown to be overexpressed on the endothelial surface overlaying active atherosclerotic plaques (53,54). In addition, experimental studies on apolipoprotein E deficient mice, which are prone to develop atherosclerotic lesions when fed a high cholesterol diet, revealed that P-selectin blockade leads to a significant decrease in mononuclear cell infiltration into atherosclerotic plaques (55). Interestingly, circulating activated platelets promote the formation of atherosclerotic lesions by facilitating the deposition of platelet-leukocyte/monocyte aggregates to the atherosclerotic endothelium in a P-selectin-dependent manner (56). This suggests that both endothelial as well as platelet expressed P-selectin contribute to the progression of atherosclerosis.

In addition to the involvement of endothelial and platelet bound P-selectin in the pathophysiology of different acute and chronic inflammatory processes, absence of P-selectin in a mouse model of anti-glomerular basement membrane glomerulonephritis led to an increased incidence of glomerulonephritis (57). Soluble P-selectin was found to be a useful diagnostic parameter in cardiovascular medicine. Increased levels of soluble P-selectin were demonstrated in patients with atherosclerosis, acute myocardial infarction, unstable angina, hypertension, and congestive heart disease (58-61). In addition, soluble P-selectin levels are s powerful predictor of future vascular events in formerly healthy women (62).

A new diagnostic approach for identifying inflamed tissue, which is high in P-selectin expression, was developed by Lindner et al. (63). They used ultrasound sensitive microbubbles bearing a monoclonal mAb against P-selectin and injected into mice subjected to renal ischemia/reperfusion. After injection, P-selectin targeted microbubbles produced a strong signal enhancement on ultrasound imaging of inflamed renal tissue (64) suggesting that this technique may be useful to assess or search for inflammation and other tissue injuries non-invasively.

Summary

P-selectin is an important leukocyte adhesion molecule mediating the initial capture and rolling of leukocytes and therefore contributing to an effective recruitment of leukocytes to sites of inflammation. In animal models, blockade of P-selectin dependent adhesion has been shown to prevent tissue injury in ischemia/reperfusion events or the progression of lesion formation in atherosclerosis. However, clinical trials have yet to confirm these promising results before patients may benefit from this therapy. In addition, it should be stressed that several differences in the physiology and pathophysiology of P-selectin exist between humans and rodents, which may lead to unexpected results in clinical trails.

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