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Inflammatory Blues Turns Velvet Skin Into Rawhide
Monocyte Rolling on Modified Endothelial PSGL-1

Rory R. Koenen, Philipp von Hundelshausen, Christian Weber Weber

The directed homing of leukocytes is crucial during immune surveillance or inflammation and is accomplished by a complex cooperation of signaling and adhesion molecules. Its pathophysiologic relevance is exemplified by various inflammatory diseases such as atherosclerosis. At present, the course of leukocyte trafficking is well established through the classical multistep cascade of initial tethering and rolling of leukocytes over vascular endothelium, followed by firm adhesion and their subsequent spreading and transendothelial migration. Leukocyte rolling is mediated by a subgroup of C-type lectins; E-, L-, and P-selectin, through shear-resistant binding to particular cell-surface carbohydrates. E- and L-selectin are expressed on activated endothelial cells and most leukocytes, respectively, while P-selectin is stored in the secretory compartments of activated platelets and endothelial cells to become rapidly upregulated on cell activation. The best characterized ligand for selectins is P-selectin glycoprotein ligand-1 (PSGL-1), a heavily posttranslationally modified homodimeric transmembrane glycoprotein. PSGL-1 contains functionally essential sialylated and fucosylated carbohydrate moieties, known as sialyl Lewis X (sLex) groups, and is expressed on blood cells such as neutrophils, monocytes, and platelets. The importance of PSGL-1 for cell recruitment is highlighted by studies using transgenic mice deficient in PSGL-1, which revealed a crucial contribution to P-selectin–dependent rolling on inflamed endothelium, indicating a function of PSGL-1 in early inflammatory responses. Presented by endothelium-bound leukocytes, PSGL-1 supports the initial L-selectin–dependent tethering of blood-borne leukocytes to the already adherent leukocytes at the inflamed vessel wall followed by E-selectin-mediated rolling on the endothelium. Besides supporting leukocyte–endothelium interactions, PSGL-1 promotes the P-selectin–dependent initial tethering of platelets to monocytes followed by more stable interactions through integrins expressed on both platelets and monocytes. In addition, the interaction between PSGL-1 and P-selectin also plays an essential role in the delivery of tissue factor to platelet aggregates via PSGL-1–bearing microparticles at sites of vascular injury. Interestingly, engagement of PSGL-1 on monocytes by platelet P-selectin has been shown to induce chemokine synthesis by monocytes, a process that depends on RANTES released by monocyte-adherent platelets. In line with this observation, activated platelets, their secretory products, and platelet-leukocyte aggregates are involved in the development of cardiovascular disease. Of particular interest in this respect is the finding that circulating activated platelets exacerbate atherosclerosis in apolipoprotein E (ApoE)-deficient mice, a process that may be attributable to the formation of platelet-monocyte aggregates, which display increased adherence to atherosclerotic endothelium both by enhanced primary and secondary tethering. In addition, activated platelets have been shown to deposit proinflammatory chemokines onto endothelium leading to increased monocyte arrest and neointima formation after injury in ApoE-deficient mice. A puzzling role in this process is played by platelet P-selectin; platelets deficient of P-selectin neither deposit RANTES nor promote neointima formation and atherosclerosis. Intriguingly, blockade of platelet PSGL-1 or endothelial P-selectin does not affect RANTES deposition, raising the question which counterligand for PSGL-1 on endothelial cells may be responsible for the proatherogenic effects of platelets. A candidate ligand would be PSGL-1, but so far studies have indicated that endothelial PSGL-1 expression is restricted to certain endothelial cell subtypes or particular (pathologic) conditions.

A possible explanation for this conundrum is provided by a study from the research group of Zwaginga published in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, which demonstrates the presence of PSGL-1 on endothelial cells. On human endothelial cells derived from umbilical vein and microvasculature, PSGL-1 was found to be constitutively expressed on the protein level, independent of inflammatory stimuli. Surprisingly, despite similar expression levels of PSGL-1 before and after stimulation with tumor necrosis factor α (TNFα), platelet adhesion and binding of a soluble P-selectin/Fc protein to activated endothelial cells was dramatically enhanced, compared with resting endothelial cells. The accumulation of platelets could be reversed by blockade of either endothelial PSGL-1 or platelet P-selectin with antibodies. These data imply that PSGL-1 on resting endothelial cells exists in a non-functional state only to become active as a cell-recruiting selectin receptor under inflammatory conditions. This notion was further supported by the observation that monocytes and platelet-monocyte aggregates only show rolling and stable interactions with TNFα-activated endothelial cells under flow conditions. Rolling and arrest of platelet-clad monocytes depended on monocyte PSGL-1 and platelet P-selectin, indicating a role for platelet P-selectin in establishing primary interactions with endothelial cells.
lial PSGL-1. However, this dependency may also reflect a possible dissociation of platelet-monocyte aggregates induced by blockade of PSGL-1 or P-selectin. Corroborative evidence for a role of endothelial PSGL-1 was provided when platelet-depleted monocytes were investigated, revealing interactions that were independent of endothelial P-selectin but rather relied on endothelial PSGL-1 and monocyte L-selectin. The function of selectin ligands depends on the correct processing of their carbohydrate structures and sulfation of sLex moieties is essential for L-selectin binding activity. In addition, stimulation by TNFα increases the expression of two enzymes involved in this sulfation. Therefore, the possibility was put forward by the authors that modification by these enzymes may be responsible for the increase in endothelial PSGL-1 activity after TNFα activation. Indeed, knockdown of one of the candidate genes, encoding the sulfotransferase GST-1/CHST1 by RNA interference led to a marked decrease of monocyte adhesion and P-selectin binding to activated endothelial cells, which was comparable with knockdown of the PSGL-1 gene. Though to a lesser extent, downregulation of the GST-2/CHST2 gene also resulted in a decrease of PSGL-1 function. Interference with two further candidate genes involved in fucosylation (FX) and glycosylation (β4GalT-7) did not have an effect on PSGL-1 function, indicating either that the encoded enzymes are not involved or that remaining low levels suffice for correct processing of PSGL-1. The observation that knock-down of GST-1 rather than GST-2 results in loss of PSGL-1 activity is in line with results from a previous study that demonstrates that sulfation of sLex by GST-1 results in stronger L-selectin binding than that mediated by GST-2. However, the direct influence of TNFα-treatment on the posttranslational processing of PSGL-1 was not investigated, leaving the question open to what extent PSGL-1 is exactly modified by GST-1 and GST-2 after inflammatory stimulation. Moreover, because the expression levels of PSGL-1 on the endothelial surface did not differ between resting and stimulated cells, the precise mechanism by which the sulfation of PSGL-1 occurs remains elusive, particularly with regard to the relatively short time-span (6 hours) observed in the present study. One potential explanation could be that PSGL-1 is continuously recycled by endothelial cells and replaced by sulfated PSGL-1 after inflammatory stimulation. Likewise, PSGL-1 may be rapidly internalized after endothelial activation to subsequently become modified and reexposed to the lumen of the vessel. Furthermore, sulfated PSGL-1 may, like P-selectin, be stored in intracellular pools. In this case, histamine or thrombin treatment would also result in increased PSGL-1-dependent monocyte interactions. It is clear that more extensive studies will have to be performed to resolve these issues.

In summary, altered posttranslational modifications of endothelial PSGL-1 during inflammatory bluses induced by mediators, such as TNF-α, results in the conversion of smooth velvet skin-like endothelium into rough rawhide (Figure). Moreover, the above findings highlight endothelial PSGL-1 as a relevant ligand for P-selectin and L-selectin supporting transient and stable interactions of monocytes and platelet-monocyte aggregates on inflamed endothelium. The presence of PSGL-1 on endothelial cells may provide an explanation for the proatherogenic platelet P-selectin–dependent interactions of platelets and platelet-derived microparticles with endothelial cells observed in previous studies. Given the importance of monocytes and platelets in cardiovascular disease, a role for endothelial PSGL-1 has to be postulated in atherosclerosis. Indeed, evidence is seeded by the authors showing that PSGL-1 is present at the luminal surface of atherosclerotic lesions of human coronary arteries. Yet, taking the constitutive expression of PSGL-1 into account, it is unclear whether a role for PSGL-1 in the pathophysiology of atherosclerosis can be inferred from this observation, because comparative specimen from healthy individuals could not be presented. Nevertheless, the intriguing possibility emerges that atheromatous PSGL-1 is highly sulfated and capable of supporting platelet and monocyte recruitment, thereby adding another relevant adhesion receptor. Extrapolating this notion, the pharmacological manipulation of carbohydrate sulfation or PSGL-1 adhesiveness may represent attractive novel approaches in the attenuation of inflammatory diseases. Until then, the
precise characterization of the role of endothelial PSGL-1 in atherosclerosis and other inflammation-related diseases would be desirable.

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None.

References