Inflammatory Blues Turns Velvet Skin Into Rawhide

Monocyte Rolling on Modified Endothelial PSGL-1

Rory R. Koenen, Philipp von Hundelshausen, Christian 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 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 P-selectin 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.

See page 1023

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 PGSL-1 and platelet P-selectin, indicating a role for platelet P-selectin in establishing primary interactions with endothelial...
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. By binding to platelet P-selectin and monocyte L-selectin, sulfated endothelial PSGL-1 facilitates rolling and stable interactions of monocytes with injured endothelium.

In summary, altered posttranslational modifications of endothelial PSGL-1 during inflammatory blues induced by mediators, such as TNF-α, results in the conversion of smooth 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 atherothrombosis. 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.

---

**Table**: Properties of PSGL-1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Platelet</th>
<th>Monocyte</th>
<th>Platelet+Monocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting</strong></td>
<td>platelet</td>
<td>monocyte</td>
<td>platelet+monocyte</td>
</tr>
<tr>
<td><strong>Flow</strong></td>
<td>platelet</td>
<td>monocyte</td>
<td>platelet+monocyte</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td>platelet</td>
<td>monocyte</td>
<td>platelet+monocyte</td>
</tr>
</tbody>
</table>

**Legend**

- P-selectin
- L-selectin
- PSGL-1

**PSGL-1 sulfation via**

- recycling/replacement?
- internalization and processing?
- intracellular pool?

---

Platelets, monocytes, and platelet-monocyte complexes do not show PSGL-1-dependent rolling on resting endothelial cells under shear flow. Through yet to be identified mechanisms, inflammatory stimuli such as TNFα lead to the expression of sulfated PSGL-1 at the endothelial surface. By binding to platelet P-selectin and monocyte L-selectin, sulfated endothelial PSGL-1 facilitates rolling and stable interactions of monocytes with injured endothelium.
precise characterization of the role of endothelial PSGL-1 in atherosclerosis and other inflammation-related diseases would be desirable.

**Source of Funding**

This work was supported by Deutsche Forschungsgemeinschaft (FOR809).

**Disclosures**

None.

**References**

Inflammatory Blues Turns Velvet Skin Into Rawhide: Monocyte Rolling on Modified Endothelial PSGL-1
Rory R. Koenen, Philipp von Hundelshausen and Christian Weber

Arterioscler Thromb Vasc Biol. 2007;27:990-992
doi: 10.1161/ATVBAHA.107.141689

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/27/5/990

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/