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Double Jeopardy

How Soluble P-Selectin Activates Leukocytes in Peripheral Arterial Occlusive Disease

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In this issue of *Circulation Research*, Woollard et al identify a critical role of pathologically elevated levels of soluble P-selectin (sP-selectin) found in the plasma of patients with peripheral arterial occlusive disease (PAOD) that promotes the activation of neutrophils and induces their adhesion to fibrinogen and platelet monolayers.[1] P-selectin is an inflammatory adhesion molecule expressed on activated platelets and endothelial cells.[2] During the last two decades, the role of inflammatory cells and adhesion molecules in the development and progression of vascular diseases has become apparent, and it is now recognized that many of the cellular and molecular events that underlie atherosclerotic vascular disease are inflammatory in nature.[3,4] One manifestation of atherosclerosis is PAOD, which is characterized by intermittent claudication and critical limb ischemia.[5] The first case of intermittent claudication caused by arterial occlusive disease was described as early as 1831 by Jean Bouley, and a few years later Heinrich Erb proposed walking as a therapy for intermittent claudication[6]; however, the precise mechanism of development of PAOD as well as the optimal treatments of this disease still remain to be determined.

Numerous studies have identified soluble inflammatory markers including acute phase proteins and adhesion molecules as risk factors for cardiovascular disease, but it is unclear whether they play causative roles as active participants in the initiation and maintenance of the inflammation (mediators) or whether they merely indicate the existence of the disease (markers). Soluble P-selectin is elevated in patients with vascular disorders such as hypertension,[7] hypercholesterolaemia,[8] unstable angina,[9] or PAOD.[10] However, until now the implications of pathophysiologic levels of sP-selectin found in the plasma of patients for the progression of these cardiovascular diseases have remained unclear.

Patients with PAOD have significantly increased levels of plasma sP-selectin (73 ng/mL compared with ≈17 ng/mL in controls). This sP-selectin exists in about equal amounts as monomers and dimers. Surprisingly, all sP-selectin seems to be present in plasma, but not in microparticles, a class of blood constituents derived from platelets, leukocytes, and endothelial, smooth muscle, and other cells. The number of platelet microparticles was previously shown to correlate with increased levels of sP-selectin in patients with peripheral artery disease.[11] The absence of P-selectin from microparticles of PAOD patients must mean that immunoreactive P-selectin is quantitatively removed from microparticles, perhaps by a proteolytic shedding mechanism previously described in platelets.[12]

A recent report showed that high concentrations of sP-selectin (>1000 ng/mL) induce an intermediate state of Mac-1 activation.[13] Mac-1 is an integrin that is involved in leukocyte adhesion to fibrinogen and other substrates,[14] but also in phagocytosis and initiation of oxygen radical production. Whereas previous work reported the effect of sP-selectin only under static conditions and did not account for possible simultaneous effects of other plasma proteins,[13] the present report extends these findings to realistic concentrations of sP-selectin and uses authentic plasma samples from PAOD patients and matched controls to analyze a role of natural forms of sP-selectin (monomer, dimer) in the induction of neutrophil adhesion.[1] Elevated levels of sP-selectin in plasma of patients with PAOD induced profound activation of neutrophils as detected by Mac-1 activation and enhanced adhesion to fibrinogen and platelets under static conditions. On platelet monolayers, this effect was also reproduced under flow conditions. Only sP-selectin, but not sE-selectin, induced adhesion to platelet monolayers. The effect of sP-selectin was shear stress–dependent and found to be more profound at low shear stress; therefore, the reported mechanism of elevated neutrophil adhesion is likely to occur in regions of low wall shear stress in vivo. The authors speculate that such shear conditions might exist in arteries of PAOD patients,[1] but it is equally possible that the findings of the present study may be relevant to diseases of the low-shear portion of the vascular system, for example, deep vein thrombosis.

sP-selectin–induced neutrophil adhesion was completely P-selectin glycoprotein ligand-1 (PSGL-1)–dependent. PSGL-1 mediates essentially all leukocyte binding to P-selectin.[15] Although the present study shows the importance of sP-selectin in the induction of elevated neutrophil adhesion to fibrinogen and activated platelets, it remains to be determined whether sP-selectin interaction with PSGL-1 expressed on monocytes and subsets of effector T lymphocytes would also lead to increased adhesion of these cells to platelets and fibrinogen, perhaps further exacerbating the development of PAOD.[16] Although elevated sP-selectin level

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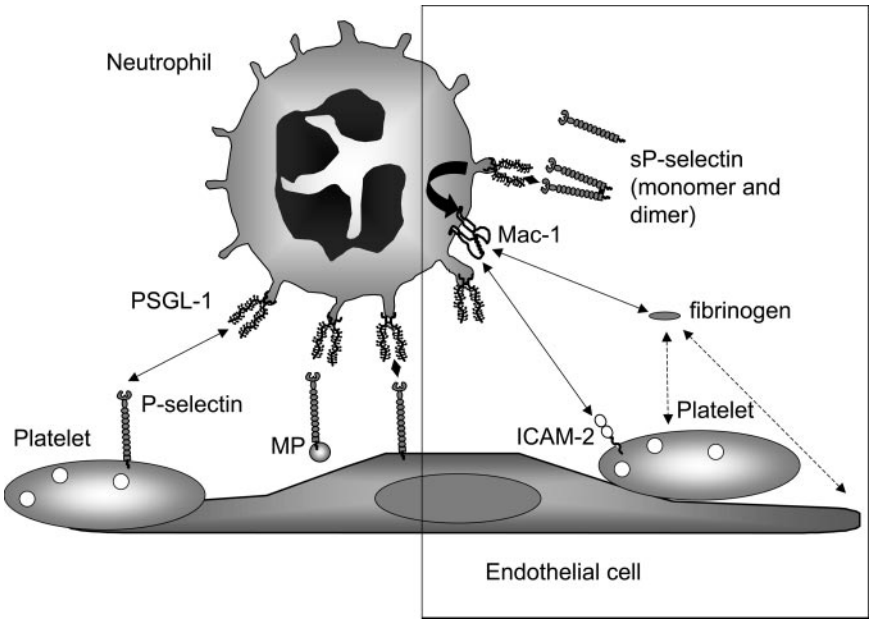

Woollard et al show that soluble P-selectin exists in plasma from PAOD patients as monomer and dimer (top right). sP-selectin binding to PSGL-1 (black antennae) on neutrophils triggers a signaling cascade (curved black arrow, details unknown, but may require src kinases) to activate Mac-1 ($\alpha_M\beta_2$ integrin heterodimer), enabling it to bind to activated platelets, presumably through ICAM-2, and to bind to fibrinogen, which can then indirectly bridge (dashed arrows) to endothelial cells (through ICAM-1, not shown) and platelets (through $\alpha_{IIb}\beta_3$ integrin, not shown). The newly demonstrated interactions (contained in box) were previously only inferred from mouse experiments. These new interactions are in addition to the P-selectin–PSGL-1 interactions known to occur between platelets, platelet-derived microparticles (MP), endothelial cells, and neutrophils (left half of figure outside box).

was found in plasma of PAOD patients, it is not clear whether plasma would be the only source of P-selectin that might affect Mac-1 activation on neutrophils in vivo. Blood from PAOD patients probably contains aggregates of neutrophils with activated platelets, which support additional mechanisms of neutrophil activation.

A possible activation and interaction cascade during the development of peripheral occlusive disease is presented in the Figure. In blood of PAOD patients, sP-selectin interacts with PSGL-1 on neutrophils with subsequent activation of Mac-1 through Src kinase–dependent mechanisms. The activation-dependent conformational change in monocyte surface Mac-1 results in fibrinogen binding to Mac-1.[17] PSGL-1 engagement by sP-selectin also leads to increased neutrophil binding to fibrinogen via Mac-1. Although the authors did not explore the molecular mechanisms of neutrophil binding to platelets, it is likely that, in addition to direct P-selectin interactions with PSGL-1, activated Mac-1 binds soluble fibrinogen, which in turn serves as a bridge by binding to platelet $\alpha_{IIb}\beta_3$ integrin.[18] All these processes promote the formation of neutrophil-platelet aggregates. A similar bridging mechanism has been described for Mac-1 binding to endothelial ICAM-1 through a fibrinogen bridge.[19] Neutrophils and monocytes activated by sP-selectin also express tissue factor.[17] In addition, sP-selectin is known to promote the formation of tissue factor–rich microparticles.[20] Both processes lead to activation of coagulation factors VII, X, and thrombin, thus initiating fibrin formation and platelet aggregation on inflamed endothelium.

As a consequence of these pathophysiological processes, PAOD patients experience a double jeopardy: not only do they have local disease in their arteries, but their plasma also contains proinflammatory activity in the form of sP-selectin, which activates neutrophils. The importance of neutrophil activation in vascular disease is supported by a study in mice, which showed that blocking neutrophil-platelet interactions resulted in significantly decreased leukocyte accumulation and reduced neointima formation after femoral artery wire injury.[21] Future studies will have to determine whether sP-selectin found in plasma of patients with hypertension, hypercholesterolemia, or unstable angina will promote similar effects, or whether the phenomena described in the present study are specific for PAOD. This current study confirms and extends the role of sP-selectin not only as a biomarker but also as an actual enhancer of PAOD progression. The present results reaffirm the possibility that blocking PSGL-1 or P-selectin by antibodies or small molecules might effectively prevent interactions of sP-selectin with circulating neutrophils and other inflammatory cells and might thus curb vascular inflammation in PAOD and perhaps other cardiovascular diseases.

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