Plasma Fibronectin Concentration
A Risk Factor for Arterial Thrombosis?

Deane F. Mosher

Accumulation and cohesion of platelets at sites of vascular injury are essential for the formation of the hemostatic plug but also result in thrombosis. Platelet plug or thrombus formation is initiated by interaction of platelet receptors with components of extracellular matrix (ECM) of injured vessels. Fibronectin is both an ECM component and moderately abundant 460-kDa glycoprotein in blood, present at 300 to 400 μg/mL (0.6 to 0.9 μmol/L) in plasma and 0.5 μg per 3×10⁸ platelets in platelet α-granules. Plasma fibronectin is a dimer of nearly identical 230-kDa subunits (Figure) that can multimerize to the insoluble form found in ECM. Because it is a ligand of platelet surface receptors and an ECM component, fibronectin has long been suspected of playing a role in platelet biology. Interestingly, fibronectin and its type I module, of which there are 12 in fibronectin (Figure), are well characterized to date only in vertebrates. Therefore, fibronectin seems to be a recent addition to the armamentarium of proteins that function in the vertebrate vasculature.

Mice homozygous for a germline disruption of fibronectin die early in embryogenesis. To study the role of plasma fibronectin in hemostasis and thrombosis, therefore, it was felt necessary to engineer mice homozygous for a “floxed” fibronectin gene in which the genes in tissues of the developed mouse could be disrupted by induced expression of cre recombinase. When the fibronectin genes in hepatocytes of these animals was disrupted, a rapid loss of plasma fibronectin occurred. Absence of plasma fibronectin did not alter the bleeding time or standard in vitro assays of hemostasis. Intravital microscopy, however, demonstrated that after injury of mesenteric arterioles, platelet thrombi build up more slowly, and groups of platelets detach and embolize from the thrombi more readily. These results indicate, therefore, that plasma fibronectin increases the stability of adherent platelet aggregates that form in response to vascular injury. Further, it has been suggested that plasma fibronectin may account for platelet thrombus formation and occlusion of injured mesenteric arterioles in mice lacking two well recognized mediators of platelet adhesion and aggregation, fibrinogen and von Willebrand factor (vWF).

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Matuskova et al now describe an interesting and potentially important new twist to this story. Mice heterozygous for the germline disruption of fibronectin, whose plasma concentrations are 50% of the normal level, had impaired formation of thrombi in mesenteric arterioles after injury with ferric chloride compared with mice with normal (100%) levels of plasma fibronectin. There was a delay in the appearance of the first thrombus, slower growth of thrombi, and constant platelet shedding. The impairment was not found after infusion of purified rat plasma fibronectin to restore plasma fibronectin concentration to the normal level, indicating that the decrease of plasma concentration to 50% of normal is responsible for the defect rather than, eg, the amount of locally derived fibronectin deposited in ECM. No impairment was noted in similarly injured mesenteric venules. The mice were otherwise normal. The mechanism of the effect was not elucidated. Interestingly, 1-μm diameter microspheres coated with plasma fibronectin were demonstrated to localize efficiently in developing arteriolar or venous thrombi.

The finding that plasma fibronectin concentration is a determinant of thrombus formation has a precedent in flow chamber studies performed by the group of Jan Sixma in the mid-1980s. These investigators separated human blood into cells and plasma, added the fractions back together, and perfused the reconstituted blood over collagen-coated surfaces. When fibronectin was depleted from the plasma by affinity chromatography on gelatin-agarose before reconstitution, platelet deposition was less, and when various amounts of fibronectin were added back platelet deposition was increased, with a dose response extending up to a concentration of 700 μg/mL (1.5 μM/L). Similarly, the concentration of plasma fibronectin is a determinant of platelet thrombus formation on surfaces coated with a matrix of fibrin or fibrin to which fibronectin was tethered covalently by activated blood coagulation factor XIII (FXIIIa). In accord with localization of the fibronectin-coated beads in developing platelet thrombi described by Matuskova et al, plasma fibronectin is deposited in developing platelet thrombi formed at moderately high shear in vitro.

By what mechanisms does plasma fibronectin stabilize and enhance platelet thrombus formation? Fibronectin has the potential to interact with platelets by 2 sites (Figure). The first is centered on module III-10 that contains the arginine-glycine-aspartic acid (RGD) sequence recognized by αIIbβ₃, αIIbβ₃, and αIIbβ₃ integrins. The second comprises the type I modules at the N termini that direct fibronectin to poorly defined sites on surfaces of adherent cells or platelets where
fibronectin is assembled into ECM fibrils. The fibronectin-coated beads studied by Matuskova et al13 presumably interact with platelets in developing thrombi via one of these sites or both. The interaction of fibronectin with \( \alpha_{IIb}\beta_3 \) on thrombin-activated platelets is half saturated at 0.3 \( \mu \text{Mol/L} \) fibronectin, ie, at about half the normal concentration of fibronectin in human plasma. Fibronectin binds to assembly sites on adherent platelets more tightly, with half saturation at a concentration of 0.05 \( \mu \text{mol/L} \), ie, 12-fold less than the normal plasma concentration.18 The dose-response data relating plasma fibronectin concentration to thrombus formation, therefore, correlate better with the dose response for RGD-mediated binding of fibronectin to activated \( \alpha_{IIb}\beta_3 \) on thrombin-activated platelets than the binding of the N-terminal region to adherent platelets. However, in the studies of the effects of fibronectin concentration on platelet thrombus formation on fibrin, both fibronectin deposition and enhancement of thrombus formation were abrogated by reagents that block binding of the N-terminal region and subsequent assembly of fibronectin by adherent platelets.17 These data suggest that it is the assembly of fibronectin into ECM-like fibrils by adherent and aggregating platelets that results in stronger cohesion of platelets and allows the thrombi to grow at the moderately high shear of the mesenteric arteriole.

The present article13 is framed around the possible consequences of decreased plasma fibronectin acutely after surgery or trauma or in patients with liver failure. However, the discussion of the significance of increased fibronectin is potentially more interesting. Studies from Korea21 and Turkey22 associated seemingly trivial increases in plasma fibronectin concentration from 360 to 380 \( \mu \text{g/mL} \) to 460 to 470 \( \mu \text{g/mL} \) with coronary artery disease. There is a considerable range in fibronectin concentrations in the adult population, with a tendency for the concentration to be higher in men than in women, increase with age, and increase in the presence of malignancy or inflammation.23–25 The present article provides an experimental rationale for trying to replicate the association between plasma fibronectin concentration and arterial disease in larger populations and learning the factors that influence the concentration. Also, as discussed in the present article13 and recently reviewed,26 studies of other genetically manipulated mouse strains have uncovered several platelet signaling pathways that are important for thrombus stability. It will be of interest to learn whether any of these pathways interact with the pathway by which fibronectin stabilizes thrombi.

References


Plasma Fibronectin Concentration: A Risk Factor for Arterial Thrombosis?
Deane F. Mosher

Arterioscler Thromb Vasc Biol. 2006;26:1193-1195
doi: 10.1161/01.ATV.0000223342.15969.7a
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 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/26/6/1193

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/