Thrombus determinants of vascular cell activation

Giorgio Ghigliotti, Paul R. Eisenberg*, Antonio Barsotti, Paolo Spallarossa, Luca Olivotti, Luca De Ferrari, Gianmarco Rosa, Claudio Brunelli

*Laboratory of Cardiovascular Biology, Department of Internal Medicine, University of Genoa, Genoa, Italy, *Lilly Research Laboratories, Indianapolis, IN, USA*

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Address:

Dr. Giorgio Ghigliotti

Dipartimento di Medicina Interna Università degli Studi Via Murcarolo, 15/1 16167 Genova E-mail: gghiglio@cardio.dimi.unige.it

Introduction

Vascular wall remodeling after arterial injury involves the migration and proliferation of smooth muscle cells (SMCs) into the intima and the development of a neointima that reduces luminal diameter. Many factors may play a role in SMC proliferation, including platelet-derived mitogens and thrombin[1,2]. The cellular events associated with neointimal proliferation in response to vascular injury may be induced in part by the platelet-rich thrombus that forms at the site of vascular injury. Platelet-rich thrombosis is dependent on a dynamic interaction between platelet adhesion and aggregation, exposure of membrane-bound tissue factor (TF), elaboration of factor Xa by the TF/VII complex, and localization of the Xa/Va complex on a phospholipid surface most likely provided by adherent platelets (Fig. 1). Platelet adhesion is mediated by specific binding of platelet membrane receptors to the subendothelial adhesive glycoproteins such as collagen, and subsequent platelet deposition is determined by several mechanisms, including extent of arterial injury, shear-dependent platelet adhesion and aggregation and elaboration of platelet agonists.

Experimental observations

We have recently shown that the factor Xa/Va complex and thrombin localize to the site of arterial injury, are resistant to physiologic anticoagulants (e.g., antithrombin III), and may promote thrombin elaboration for up to 5 days after injury of normal vessels in New Zealand White rabbits[3]. The activity of the Xa/Va complex that localizes to the injured arterial wall is regulated by platelet deposition after injury, providing a membrane for assembly of the complex, and TF-mediated activation of factor X. As TF is critical for the initiation of coagulation cascade, we created a model where a sequential injury to the ab-

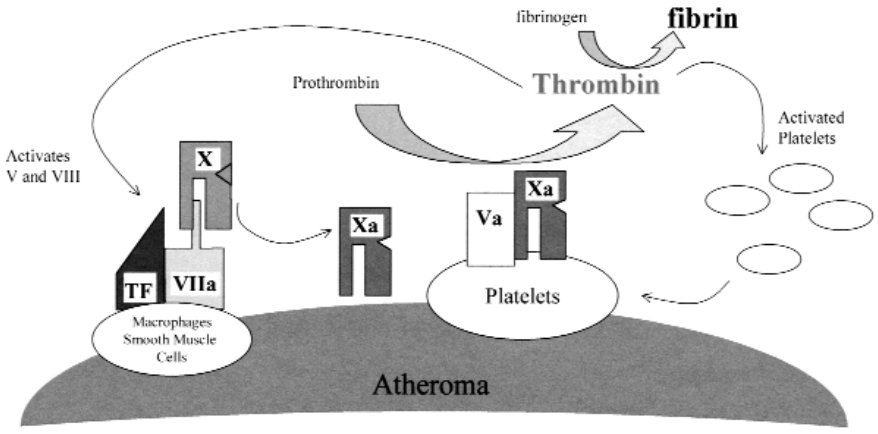

Figure 1. *Central role of the prothrombinase enzymatic complex. TF = tissue factor.*

dominal aorta of New Zealand White rabbits was used, to define whether the subendothelium and the media of an aorta with neointima, 30 days after a first catheter overinflation, expose TF, and to define also the time course after arterial injury of the increase in procoagulant activity and its determinants on the luminal surface. We evaluated also whether this activity was associated with a more robust and more prolonged procoagulant activity compared to that in a normal aorta. Procoagulant activity dependent on TF and platelets was evaluated at 4 hours after a repeated injury, while procoagulant activities dependent on factor Xa/Va and thrombin were also evaluated at longer intervals after a repeated injury. Prothrombotic activity was characterized *ex vivo* in injured segments that were incubated with barium-adsorbed plasma and with chromogenic substrates to define the presence of procoagulant activity bound to the vessel wall. Animals with neointima showed increased platelet adhesion 4 hours after injury, when compared to animals with normal vessels, suggesting availability of upstream activators of the prothrombinase complex. At the same interval, functional activity of complexes inducing activation of factor X, evaluated *ex vivo* with chromogenic substrates, was detectable in animals with neointima, and was consistently reduced (81%) after incubation of the injured segments with 2.5 mg/ml of a specific antibody to rabbit TF. Animals with neointima showed increased platelet adhesion 4 hours after injury, suggesting availability of upstream activators of the prothrombinase activity. Thus, in our experimental conditions, platelet coverage and procoagulant activity were detected after repeated injury of the abdominal aorta of rabbits. Since injured segments of aorta with the neointima expressed capacity to support prothrombinase activation, as indicated by activation of factor X from upstream active coagulation complexes, and because a TF antibody was effective in reducing activation of factor X in the injured vessels, our data suggest that exposure of TF mediates an increased prothrombotic activity associated with intimal hyperplasia. This finding suggests that cells in the neointima support activation of the coagulation mediated by TF: once intimal TF is exposed to the circulating blood, it results in a rapid fibrin deposition. Definite candidates for supporting TF activity in the neointima and the media are vascular SMCs and monocytes coming from the circulating blood.

Regulation of vascular inflammation by procoagulant activity

Differently from *in vitro* experiments, whose focus is to define mechanisms by which thrombosis and inflammation act unidirectionally with respect to endothelial cell or leukocyte function, experimental preparations *in vivo* may reveal important crosstalk between cell populations which may influence how each cell population responds to a stimulus, especially if factors are present that activate both populations. Thus, thrombin and other procoagulant moieties (Xa/Va complex) may act in a synergistic fashion with inflammatory cytokines (interleukin-4, interleukin-6) to induce functional expression of adhesion molecules critical for recruitment of inflammatory cells to the vascular wall or to interact with monocytes to potentiate recruitment of additional inflammatory cells. The extent and the role to which platelet-derived and coagulation-derived factors act separately or in concert *in vivo* to induce activation of SMCs and endothelial cells are not known. As inhibition of platelet-rich thrombosis attenuates vascular wall remodeling in experimental models, and because clinical events associated with restenosis after angioplasty have been shown to be attenuated by inhibition of the platelet fibrinogen receptor, it was postulated that inhibition of platelet activation, of thrombin, or of procoagulants that induce thrombin elaboration, also attenuates stenosis after vascular injury. Tanaka et al.[4] demonstrated an increased expression of VCAM and ICAM 48 hours after balloon denudation of the endothelium in rabbits: VCAM expression appears closely associated with the leading edge of regenerating endothelial cells, and high levels of ICAM-1 expressions were seen in both the endothelial and SMCs of the developing neointima. These adhesion molecules serve as specific mediators of leukocyte recruitment, retention and activation following arterial injury, thus serving as ideal early markers of the response to vascular injury[5].

Conclusions

Since in experimental models it is possible to modulate the procoagulant and platelet responses to arterial wall injury with the use of specific inhibitors of thrombin, factor Xa, TF/VIIa, and platelet adhesion and aggregation, characterization of the expression of VCAM and ICAM will provide insight to address the following issues: 1) relevance of biological extracoagulative role of prothrombotic moieties, 2) establishing whether endothelial and SMC activation early after injury are procoagulant and/or platelet-dependent.

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