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## ● ● ● THROMBOSIS & HEMOSTASIS

Comment on Robins et al, page 692

# Gas6 gains entry into the coagulation cascade

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In this issue of *Blood*, Robins and colleagues provide new insights into how Gas6 promotes thrombosis through contributions from platelets and from the vascular wall. By showing that Gas6 up-regulates the initiator of coagulation, tissue factor, in the endothelium, these studies may yield new and safer treatments for thrombotic disease.<sup>1</sup>

**G**as6 is a vitamin K dependent (VKD) protein, a member of a family that includes coagulation factors II, VII, IX, and X, protein C, protein S, and protein Z.<sup>2</sup> Despite its structural relationship to these critical components of the clotting system and genetic data suggesting that Gas6 participates in vascular disease, a defined role for Gas6 in fibrin clot formation has remained elusive. Here, Robins et al narrow that gap in our knowledge.

Gas6 is the ligand for members of the TAM family of receptor tyrosine kinases (RTKs), Tyro, Axl, and Mer. These single transmembrane RTKs contain a cytoplasmic tyrosine kinase domain that is autophosphorylated when the receptor dimerizes in response to Gas6 binding to its extracellular domain. Axl, the best characterized, has the highest affinity for Gas6 and like Gas6, is expressed by many cells, including vascular smooth muscle cells, endothelial cells, platelets, monocytes, and bone marrow cells. Gas6-TAM binding induces many biologic effects, promoting reversible growth arrest, cell survival, prolifera-

tion, migration, and adhesion (reviewed in Laurance et al<sup>3</sup>). Most responses are mediated via activation of phosphatidylinositol-3-kinase (PI3-kinase), although other pathways have been implicated, adding to the complexity of evaluating the role(s) of Gas6 in health and disease.

Major insights into the pathophysiologic relevance of Gas6, particularly in the vasculature, have been gained through studies using genetically engineered mice. In mouse models of arterial injury or atherosclerosis, deficiency of Gas6 or Axl results in vasculoprotection, with reduced intimal media thickening and smaller, noninflammatory, stable plaques.<sup>4</sup> Most notably, these mice are protected against venous and arterial thrombosis.<sup>5</sup> Until the report by Robins and colleagues this was attributed entirely to a Gas6-dependent platelet function defect.

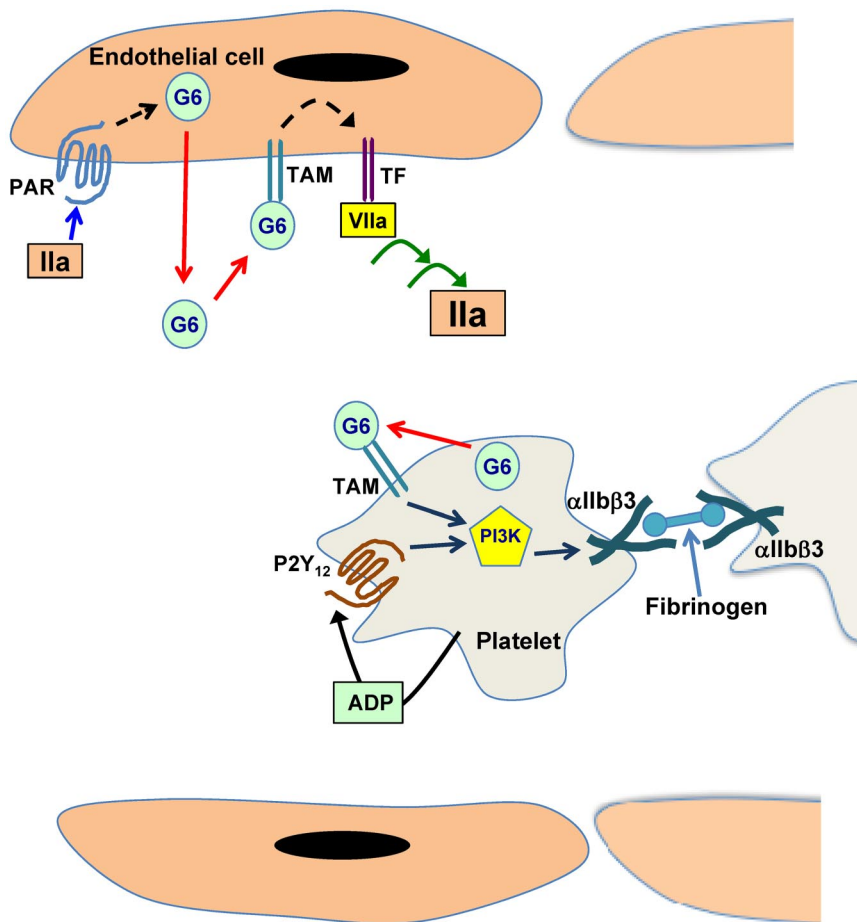
In response to injury, damaged endothelium promotes recruitment, adhesion, activation, and aggregation of platelets.<sup>6</sup> Fibrinogen facilitates platelet aggregation and clot retrac-

tion through engagement of the integrin  $\alpha$ IIB $\beta$ 3. For this to occur the integrin must be activated, whereupon it undergoes a conformational change that allows it to interact with fibrinogen. ADP-mediated platelet activation is one route by which this is achieved. ADP, however, does not act alone, and additional agonists appear to be required to sustain integrin activation. Gas6 is believed to contribute to  $\alpha$ IIB $\beta$ 3 activation and platelet aggregation by synergizing with ADP through a TAM-mediated PI3-kinase-dependent pathway (see figure).<sup>7</sup> As one might expect, platelets that lack Gas6 have a defect in ADP-induced aggregation. However, this defect is subtle,<sup>5</sup> and by itself may not explain the protection afforded Gas6-null mice from thrombosis. Robins et al sought alternative explanations.

They hypothesized that Gas6 from the vascular wall plays an important role in the pathophysiology of venous thromboembolism. This was tested via elegant approaches. To distinguish the contribution of Gas6 from hematopoietic and nonhematopoietic compartments, they generated chimeric mice using bone marrow transplantation between wild-type and Gas6-deficient mice. Animals lacking Gas6 in both compartments had smaller thrombi, while those with Gas6 in one or the other compartment had intermediate-sized thrombi, significantly smaller than in wild-type mice. Supported by platelet depletion/reconstitution experiments, they reasonably concluded that Gas6 derived from the hematopoietic and nonhematopoietic compartments, that is, platelets and the vessel wall, both contribute to venous thrombosis. What, then, is the mechanism by which the vasculature contributes to clotting in a Gas6-dependent manner?

Although Gas6 is known to activate endothelial cells, partly by increasing leukocyte adhesion molecule expression,<sup>8</sup> no one had previously considered that the initiator of coagulation, tissue factor, might be regulated by Gas6. Robins and colleagues showed that Gas6-null mice exhibit an almost complete absence of tissue factor in the wall of the damaged vessel, in contrast to that of wild-type mice. Although the source of the tissue factor, endothelial versus perivascular cells, was not ascertained from their studies, they assessed the relationship between Gas6 and tissue factor in endothelial cells in vitro.

In response to thrombin stimulation, Gas6-null endothelial cells expressed less cell



Gas6 (G6) exerts prothrombotic properties in the hematopoietic and vascular compartments. Shown is a highly simplified cartoon model of effects of Gas6 on platelets and endothelial cells. As platelets adhere and activate on contact with damaged endothelial cells (not shown), platelet aggregation is achieved through synergistic intracellular signaling events that are mediated by Gas6-TAM and ADP-P2Y<sub>12</sub> interactions. These induce activation of PI3-kinase that in turn, leads to sustained activation of  $\alpha$ IIb $\beta$ 3 and engagement with fibrinogen. Thrombin (IIa), presumably acting via one of the protease activated receptors (PAR), induces the release of Gas6 from endothelial cells by unknown mechanisms. Gas6 that is released (or circulating) subsequently binds to one of the TAMs, which leads to up-regulation of tissue factor (TF), again via unknown mechanisms. TF can then initiate coagulation, first by binding to factor VIIa, ultimately leading to further thrombin generation and the formation of a fibrin clot (not shown). Relative sources of Gas6 have not been elucidated, and there is likely cross-talk between cells. The possible contribution of smooth muscle cells and other circulating cells is not shown.

surface tissue factor than wild-type endothelial cells. Remarkably, exogenous Gas6 restored the response of Gas6-null endothelial cells to express tissue factor. This suggested that soluble Gas6, either exogenous (eg, plasma-derived) or secreted in response to an agonist such as thrombin, induces intracellular signals, presumably via TAMs, to up-regulate tissue factor in the vasculature (see figure). It will be important to determine whether these pathways are active in perivascular cells and/or in monocytes. Nonetheless, the findings finally establish a link between Gas6 and the coagulation cascade.

Which Gas6-TAM signaling pathways are involved in regulating tissue factor? Gas6-TAM interactions most profoundly activate PI3-kinase, and PI3-kinase inhibitors are holding promise as antithrombotic agents.<sup>9</sup> However, in most cells, including endothelial and smooth muscle cells, activation of PI3-kinase suppresses tissue factor. Thus, this is not likely the pathway by which Gas6 increases tissue factor. But as noted before, multiple signaling pathways participate in cellular responses to Gas6, and it will take some work to tease out which ones specifically modulate tissue factor expression. The findings, how-

ever, are intriguing and raise the question as to how the TAMs participate in regulating tissue factor. It will also be important to assess the roles of perivascular cells and monocytes in Gas6-TAM-mediated activation of coagulation.

Overall, the curiosity of Robins et al that drove them to explain a gap between in vitro and in vivo findings has finally placed Gas6 into the coagulation pathway, along with the other VKD proteins. Although mechanisms are still to be delineated, the findings hold promise for the development of new strategies to prevent thrombosis. Interfering with Gas6 and/or the TAMs may result in decreased platelet aggregation, clot retraction, and tissue factor expression, thereby limiting thrombus growth while maintaining a low risk of bleeding.

*Conflict-of-interest disclosure:* The authors declare no competing financial interests. ■

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