GAS6
Pouring GASoline Into the Inflammatory Inferno of Venous Thrombosis
Christoph Reinhardt

Venous thrombosis is determined by the recruitment of monocytes and neutrophils to the inflamed endothelium and is primarily influenced by the plasmatic coagulation system. Monocyte tissue factor (TF) was identified as the causative trigger for intraluminal fibrin formation and thrombus load in the inferior vena cava (IVC) stenosis model, resembling human deep vein thrombosis. Although monocyte TF is prothrombotic, the TF expressed by activated endothelial cells triggers proinflammatory protease-activated receptor signaling pathways.

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In the past years, GAS6 (growth arrest–specific gene-6) was described as a major regulatory protein of prothrombotic signaling (Table). Clinically, elevated plasma GAS6 levels were associated with venous thromboembolic disease. In previous work, the Blostein laboratory has identified GAS6, the major ligand of the TAM (Tyro3/Axl/Mer) receptor tyrosine kinase Axl, that protects endothelial cells from apoptosis via P13K (phosphoinositide 3-kinase)-Akt–dependent inactivation of FOXO1a, as a stimulant of Akt-mediated endothelial TF expression. Mice deficient in GAS6 or the GAS6 receptors are protected against thrombosis. However, given that GAS6 derived from the hematopoietic and the nonhematopoietic vascular compartment contributes equally to thrombus formation, defective thrombus formation in mice deficient in GAS6 signaling may have several reasons. A platelet phenotype, characterized by impaired dense and α-granule release, impaired platelet aggregation, and retarded spreading on fibrinogen coatings, became apparent in these mice. The platelet defect was explained by the regulatory role of GAS6 receptors in platelet outside-in signaling via the αIIbβ3 integrin, exerting a synergistic effect on the P2Y12-mediated ADP signaling pathway, prolonging Akt phosphorylation. Importantly, in addition to impaired platelet function, GAS6 deficiency also showed an endothelial phenotype. The characterization of isolated endothelial cells from GAS6-deficient mice revealed a reduction of thrombin-induced FOXO1-dependent vascular cell adhesion molecule-1 expression, a relevant adhesion molecule supporting monocyte–endothelial interactions. Furthermore, in a model of cancer-induced thrombosis, GAS6 was required for cancer-related upregulation of platelet-activating prostaglandin E2 in lung endothelial cells.

Because the nature of thrombus formation varies tremendously in different vascular beds and also depends on the applied thrombosis model, it is conceivable that GAS6 may have additional prothrombotic signaling functions, for example, related to innate immune cell recruitment. Because the ferric chloride injury model has been demonstrated to lead to increased expression and procoagulant activity of TF in the IVC vessel wall, it is instrumental to apply the flow restriction model of the IVC, in which thrombus formation was demonstrated to be independent of vessel wall TF, but predominantly depends on myeloid cell–derived TF.

In this issue of ATVB, Laurance et al have shown in a stenosis mouse model of the IVC that GAS6 is a relevant player in the recruitment of inflammatory CCR2^hiCX3CR1^lo monocytes, determining thrombus size. Complementary, this effect of GAS6 on monocyte recruitment was also shown in the ferric chloride injury model, which in addition to other exposed prothrombotic stimuli is strongly dependent on vessel wall TF. In contrast, recruitment of neutrophils was unaffected by GAS6 deficiency. Although the proportion in CCR2^hiCX3CR1^lo monocytes was reduced in GAS6-deficient mice, the CX3CR1^hi subset remained stable, pointing to a distinct role of proinflammatory CCR2^hiCX3CR1^lo monocytes in the process of deep vein thrombosis. The authors demonstrated that monocyte depletion reduced thrombus size, and thrombus size was specifically dependent on the presence of the CCR2 (C-C chemokine receptor 2)–positive monocyte subset and related CCL2 (CC-chemokine ligand 2; MCP-1 [monocyte chemotactic protein-1]) signaling. Consequently, because myeloid cells are an important source of procoagulant TF, determining thrombus weight in this deep vein thrombosis model, Laurance et al specifically pinpointed the impact of thrombin signaling through protease-activated receptor-1, demonstrating the requirement of GAS6 for the productive expression of CCL2 in bone marrow–derived monocytes and endothelial cells. Conditioned medium from thrombin-stimulated endothelial cells augmented wild-type monocyte migration but not if the monocytes were incubated with conditioned medium from GAS6-deficient mice or with medium from wild-type cells that was pre-treated with a CCL2-blocking antibody. In support of the critical role of P-selectin for leukocyte accumulation in deep vein thrombosis, the group identified CCL2 in endothelial cells and CCR2 on monocytes as the critical thrombin-induced factors, enhancing the expression of the P-selectin counter-receptor P-selectin glycoprotein ligand-1 on monocytes. More specifically, they found that

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GAS6 increases CCL2 expression in endothelial cells via the c-Jun N-terminal kinase pathway, assisting monocyte migration. Altogether, the authors provide compelling evidence for the implication of GAS6 in the recruitment of proinflammatory CCR2hiCX3CR1lo monocytes and its involvement in venous thrombosis (Figure).

The work of Laurance et al in this issue of ATVB defines the endothelial GAS6–CCL2 signaling axis as a pivotal element of venous thrombogenesis. Laurance et al have uncovered a novel regulatory role for GAS6 in IVC thrombosis that favors thrombin-dependent protease-activated receptor-1 activation in the endothelium, enhancing endothelial CCL2 expression and CCR2-mediated augmentation of proinflammatory CCR2hiCX3CR1lo monocyte adhesion, which they defined as the relevant key subset. Because previous work has elaborated the role of myeloid TF for thrombus growth in the IVC flow restriction model but did not find impaired leukocyte adhesion in the low-human TF mouse

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<td>Laurance et al14</td>
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CCL2 indicates CC-chemokine ligand 2; CCR2, C-C chemokine receptor 2; CX3CR1, CX3C chemokine receptor 1; GAS6, growth arrest–specific gene-6; IVC, inferior vena cava; PAR, protease-activated receptor; PSGL-1, P-selectin glycoprotein ligand-1; TF, tissue factor; and VCAM-1, vascular cell adhesion molecule-1.

**Figure.** GAS6 (growth arrest–specific gene-6) promotes PAR1 (protease-activated receptor-1)–dependent thrombin signaling to induce CCL2 (CC-chemokine ligand 2) expression in the flow-restricted inferior vena cava (IVC) endothelium, driving the recruitment of CCR2hiCX3CR1lo monocytes to support thrombus growth. Dependent on GAS6, thrombin signaling induces CCL2 in endothelial cells and CCR2 (C-C chemokine receptor 2) on monocytes, propagating an endothelial monocytic signaling axis that determines the size of nascent IVC thrombi, potentially through the upregulation of PSGL-1 (P-selectin glycoprotein ligand-1) expression and a pronounced interaction with P-selectin. The relevant sources of GAS6 and the procoagulant stimuli resulting in signaling active thrombin in situ, as well as the relevant receptor interactions on the endothelium are to be defined. CX3CR1 indicates CX3C chemokine receptor 1.
model, further experimental efforts are needed to elucidate the sources of TF that potentially contribute to thrombin generation. The signaling-relevant localization of this chameleon-like protease to IVC endothelial cells in situ by specific receptors may strongly impact its signaling function in various diseases. Considering the recently demonstrated function of the coagulation-vascular circuit triggering angiotensin II–induced vascular dysfunction mediated by coagulation factor XI feedback via the interaction of the glycoprotein 1bα receptor with thrombin on platelets, it will be highly interesting to explore whether the uncovered endothelial-localized GAS6-dependent signaling mechanism is supported by the coagulation factor XI feedback. This platelet-localized factor XI–dependent mechanism promotes leukocyte infiltration in the arterial system, likewise inducing CCL2 and vascular coagulation factor XI feedback. This platelet-localized factor XI–dependent mechanism is supported by the testing to explore whether the uncovered endothelial-localized GAS6-dependent signaling mechanism is supported by the coagulation factor XI feedback. Gas6-dependent signaling mechanism is supported by the testing to explore whether the uncovered endothelial-localized GAS6-dependent signaling mechanism is supported by the coagulation factor XI feedback.

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Disclosures
None.

References
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