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.

See accompanying article on page 1315

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 PI3K (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 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 CCR2hiCX3CR1lo 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 CCR2hiCX3CR1lo monocytes was reduced in GAS6-deficient mice, the CX3CR1hi subset remained stable, pointing to a distinct role of proinflammatory CCR2hiCX3CR1lo 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 CX3CR1hi subset. 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 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.
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 al14 in this issue of *ATVB* defines the endothelial GAS6–CCL2 signaling axis as a pivotal element of venous thrombogenesis. Laurance et al14 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
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 Ibα 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 cell adhesion molecule-1 expressions in the vessel wall. It will be intriguing to see how future research will explain the biochemical mechanism of how the γ-carboxyglutamate acid-rich (Gla) domain containing protein GAS6 acts in concert with thrombin receptor (protease-activated receptor-1) signaling on endothelial cells. Furthermore, the relevant vascular sources of signaling-relevant GAS6 and the relevant receptor interactions await further investigation in the setting of venous thrombosis. In the future, a detailed understanding of GAS6 in this signaling route may potentially allow for therapeutic intervention to combat venous thrombosis.

### Sources of Funding

This research is supported by the German Federal Ministry for Education and Research (BMBF 01EO1003 and BMBF 01EO1503), the DFG (Deutsche Forschungsgemeinschaft; RE 3450/3-1), the Else Kröner-Fresenius-Stiftung (2014-A151), and the Boehringer Ingelheim Foundation.

### Disclosures

None.

### References


**Key Words:** Editorials ◼ CC-chemokine ligand 2 ◼ endothelial cells ◼ growth arrest-specific gene-6 ◼ thrombin ◼ vascular cell adhesion molecule-1 ◼ vena cava, inferior ◼ venous thrombosis
GAS6: Pouring GASoline Into the Inflammatory Inferno of Venous Thrombosis
Christoph Reinhardt

Arterioscler Thromb Vasc Biol. 2017;37:1263-1265
doi: 10.1161/ATVBAHA.117.309585
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 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/37/7/1263

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/