

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.¹ 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.¹ Although monocyte TF is prothrombotic, the TF expressed by activated endothelial cells triggers proinflammatory protease-activated receptor signaling pathways.^{2,3}

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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.¹⁰ 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,^{7,9} as a stimulant of Akt-mediated endothelial TF expression.¹² Mice deficient in GAS6 or the GAS6 receptors are protected against thrombosis.^{4,5} However, given that GAS6 derived from the hematopoietic and the nonhematopoietic vascular compartment contributes equally to thrombus formation,¹¹ defective thrombus formation in mice deficient in GAS6 signaling^{4,5} 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 $\alpha_{IIb}\beta_3$ integrin,⁵ exerting a synergistic effect on the P₂Y₁₂-mediated ADP signaling pathway, prolonging Akt phosphorylation.⁶ 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.¹³ Furthermore, in a model of cancer-induced thrombosis, GAS6 was required for cancer-related upregulation of platelet-activating prostaglandin E2 in lung endothelial cells.¹⁵

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,¹¹ 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.¹

In this issue of *ATVB*, Laurance et al¹⁴ have shown in a stenosis mouse model of the IVC that GAS6 is a relevant player in the recruitment of inflammatory CCR2^{hi}CX3CR1^{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.¹⁶ In contrast, recruitment of neutrophils was unaffected by GAS6 deficiency. Although the proportion in CCR2^{hi}CX3CR1^{lo} monocytes was reduced in GAS6-deficient mice, the CX3CR1^{hi} subset remained stable, pointing to a distinct role of proinflammatory CCR2^{hi}CX3CR1^{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 chemoattractant protein-1]) signaling. Consequently, because myeloid cells are an important source of procoagulant TF, determining thrombus weight in this deep vein thrombosis model,¹ Laurance et al¹⁴ 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,¹ 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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Table. Milestones of Discoveries on the Signaling Function of GAS6 in Thrombosis

Year	Major Discoveries	References
2001	Lack of GAS6 function results in platelet dysfunction and is protective against thrombosis because of defective thrombus stabilization.	Angelillo-Scherrer et al, ⁴ Angelillo-Scherrer et al, ⁵ Cosemans et al ⁶
2002	CCL2 expression is related to PAR1, but not PAR2 signaling in endothelial cells.	Riewald et al ⁹
2004	Role of γ -carboxylated GAS6 as a survival factor of endothelial cells.	Hasanbasic et al, ⁷ Hasanbasic et al, ⁸ Ganopolsky et al ⁹
2011	Plasma GAS6 levels are associated with venous thromboembolic disease.	Blostein et al ¹⁰
2014	GAS6 signaling via Axl induces TF expression in endothelial cells, supporting venous thrombosis.	Robins et al, ¹¹ Laurance et al ¹²
2015	GAS6 regulates the thrombin-induced expression of endothelial adhesion receptors VCAM-1.	Bertin et al ¹³
2017	GAS6 regulates thrombin signaling on endothelial cells supporting CCL2-mediated recruitment of proinflammatory CCR2 ^{hi} CX3CR1 ^{lo} monocytes by increasing PSGL-1 expression, thus augmenting thrombus growth in the IVC thrombosis.	Laurance et al ¹⁴

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.

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 CCR2^{hi}CX3CR1^{lo} 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 CCR2^{hi}CX3CR1^{lo} 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

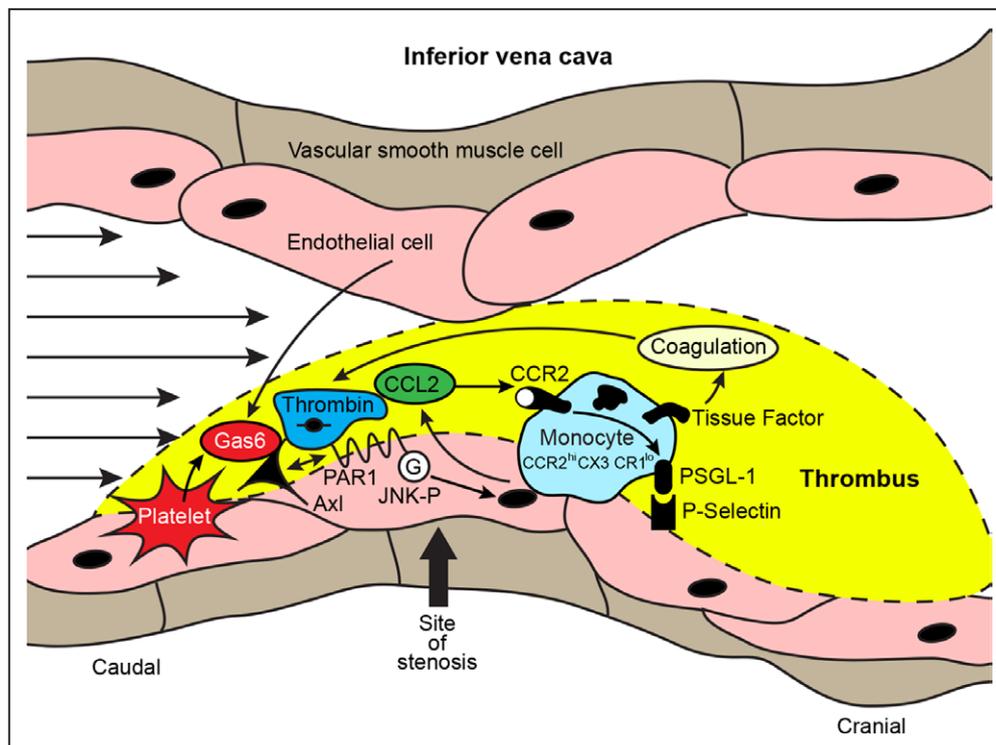


Figure. GAS6 (growth arrest–specific gene-6) promotes PAR1 (protease-activated receptor-1)–dependent thrombin signaling to induce CCL2 (CC-chemokine ligand 2) expression in the flow-restricted inferior vena cava (IVC) endothelium, driving the recruitment of CCR2^{hi}CX3CR1^{lo} monocytes to support thrombus growth. Dependent on GAS6, thrombin signaling induces CCL2 in endothelial cells and CCR2 (C-C chemokine receptor 2) on monocytes, propagating an endothelial monocyte 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,¹ 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 1b α receptor with thrombin on platelets,¹⁷ 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.¹⁷ It will be intriguing to see how future research will explain the biochemical mechanism of how the γ -carboxyglutamic acid-rich (Gla) domain containing protein GAS6⁸ 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.

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Disclosures

None.

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