

Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms

Shaun P. Jackson,^{1,3,*} Roxane Darbousset,^{1,2,*} and Simone M. Schoenwaelder^{1,2,*}

¹Heart Research Institute, Newtown, NSW, Australia; ²Charles Perkins Centre, The University of Sydney, Camperdown, NSW, Australia; and ³Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA

Thrombosis with associated inflammation (thromboinflammation) occurs commonly in a broad range of human disorders. It is well recognized clinically in the context of superficial thrombophlebitis (thrombosis and inflammation of superficial veins); however, it is more dangerous when it develops in the microvasculature of injured tissues and organs. Microvascular thrombosis with associated inflammation is well recognized in the context of sepsis and ischemia-reperfusion injury; however, it also occurs in organ transplant rejection, major trauma, severe burns, the antiphospholipid syndrome, preeclampsia, sickle cell disease, and biomaterial-induced thromboinflammation. Central to thromboinflammation is the loss of the normal antithrombotic and anti-inflammatory functions of endothelial cells, leading to dysregulation of coagulation, complement, platelet activation, and leukocyte recruitment in the microvasculature. α -Thrombin plays a critical role in coordinating thrombotic and inflammatory responses and has long

been considered an attractive therapeutic target to reduce thromboinflammatory complications. This review focuses on the role of basic aspects of coagulation and α -thrombin in promoting thromboinflammatory responses and discusses insights gained from clinical trials on the effects of various inhibitors of coagulation on thromboinflammatory disorders. Studies in sepsis patients have been particularly informative because, despite using anticoagulant approaches with different pharmacological profiles, which act at distinct points in the coagulation cascade, bleeding complications continue to undermine clinical benefit. Future advances may require the development of therapeutics with primary anti-inflammatory and cytoprotective properties, which have less impact on hemostasis. This may be possible with the growing recognition that components of blood coagulation and platelets have prothrombotic and proinflammatory functions independent of their hemostatic effects. (*Blood*. 2019;133(9):906-918)

Thrombosis and inflammation

The coordinated activation of inflammatory and hemostatic responses following infection or tissue injury is a phylogenetically conserved defense mechanism that can be traced back to early invertebrates.¹ In these primitive organisms, a single cell (the hemocyte) can perform basic inflammatory, immune, and hemostatic functions.¹ However, most higher-order mammals have evolved a complex multicellular system encompassing platelets and a variety of leukocyte subsets, including neutrophils, monocytes, and a series of antigen-presenting cells. Similarly, some of the major humoral components of innate immunity have evolved from a rudimentary complement system into a sophisticated series of highly integrated protease cascades, including the complement, coagulation, fibrinolysis, and contact-kinin systems. Despite this close evolutionary development, thrombosis and inflammation have traditionally been viewed as distinct complementary processes. Thrombosis can be best defined as an exaggerated hemostatic response, leading to the formation of an occlusive blood clot obstructing blood flow through the circulatory system. By comparison, inflammation is the term applied to the complex protective

immune response to harmful stimuli, such as pathogens, damaged cells, or irritants. However, with the recognition that inflammation stimulates thrombosis, and in turn, thrombosis promotes inflammation, the functional interdependence of these processes has become increasingly well defined (Figure 1A).

A growing body of evidence indicates that some autoimmune disorders, such as rheumatoid arthritis and systemic lupus erythematosus, are regulated by components of the hemostatic system.² Thrombotic disorders, such as paroxysmal nocturnal hemoglobinuria or atypical hemolytic uremic syndrome, are triggered by humoral components of innate immunity (complement), and many microvascular inflammatory disorders promote platelet activation and coagulation. This is most clearly demonstrated in the acute exaggerated thromboinflammatory responses that accompany sepsis, ischemia-reperfusion (IR) injury, and major trauma. In these latter disorders, the ultimate extent of organ injury is dependent on the primary insult (bacterial invasion, organ ischemia, or traumatic injury), as well as on the extent of the ensuing microvascular thromboinflammatory

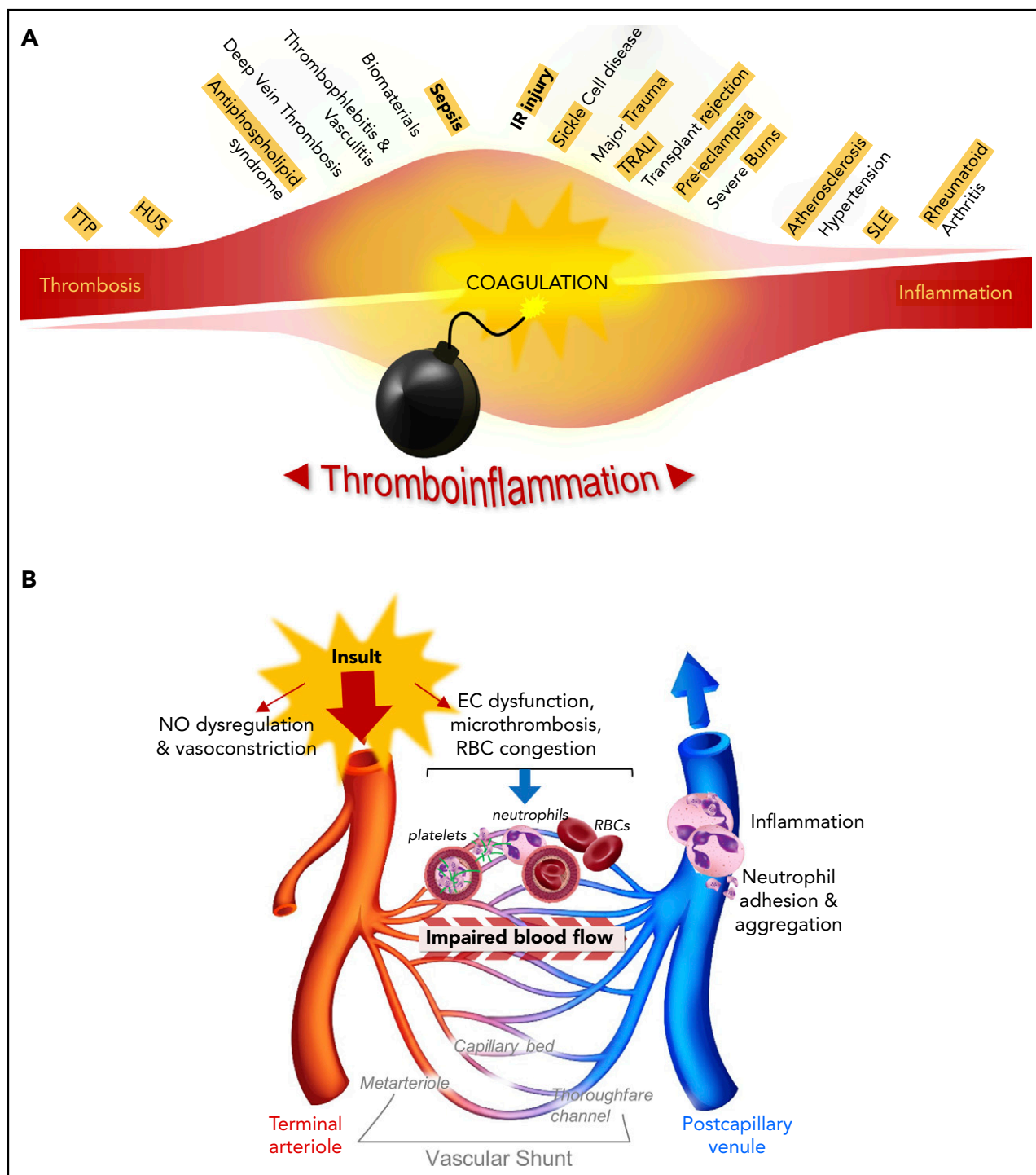


Figure 1. Thromboinflammation: an important pathogenic process linked to a diverse range of human diseases. (A) A broad spectrum of human disorders is associated with thromboinflammatory complications, many of which have microvascular dysfunction. It is likely that the level of α -thrombin generation is a key determinant of the extent of the thromboinflammatory response. For example, diseases such as sepsis, ischemia reperfusion (IR) injury (the focus of this review), and organ transplant rejection are associated with widespread activation of coagulation throughout the microcirculation, which is accompanied by an intense thrombotic and inflammatory response. At the extremes of this spectrum, the microvascular thrombotic disorder thrombotic thrombocytopenia purpura (TTP) exhibits limited α -thrombin generation and is associated with a limited inflammatory response in the early phase of the disease. At the other end of the spectrum, autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus (SLE), are primarily considered inflammatory disorders, with a limited role for α -thrombin. Nonetheless, other hemostatic components appear to contribute to the pathogenesis of these diseases. (B) Tissue or organ injury by diverse pathogenic mechanisms is commonly associated with microvascular thromboinflammatory responses. Microvascular obstruction can be mediated by all cellular elements in blood, including platelets, neutrophils, and red blood cells (RBCs), with stable occlusion typically linked to the activation of coagulation and fibrin generation. Regardless of the primary insult to tissues or organs (infection, ischemia, or trauma), the ultimate outcome of these disorders is heavily influenced by the extent of the microvascular thrombotic and inflammatory responses. EC, endothelial cell; HUS, hemolytic-uremic syndrome; NO, nitric oxide; TRALI, transfusion-related acute lung injury.

response (Figure 1B). When severe, thrombin generation can extend systemically and damage remote organs, particularly the lung (acute lung injury or acute respiratory distress syndrome) and kidneys (acute kidney injury). This is a major issue in critically ill patients and can lead to the development of multiorgan dysfunction syndrome and death. Thus, defining the molecular mechanisms regulating thrombin generation in specific disease states is of major clinical importance. The primary focus of this review will be on the role of coagulation in promoting acute microvascular thrombosis and in generation during sepsis and IR injury. These disorders have been most intensively investigated in the context of thrombin generation, and much has been learned from sepsis and IR injury about the potential benefits and limitations of targeting coagulation processes in the clinic. For more detailed information on the interaction between coagulation and other components of the hemostatic and innate immune system, several excellent review articles have been published.³⁻⁷

Endothelium: a critical regulator of thrombin generation

The endothelium lines the lumen of the entire circulatory system, from the chambers of the heart down to the microcapillary beds. However, quantitatively, ~98% of all endothelial cells reside within the microvasculature, a reflection of the vast surface area of the microcirculatory system in the human body.^{8,9} Endothelial cells maintain vascular health by exerting antiplatelet, anticoagulant, and anti-inflammatory actions (Figure 2). Quiescent endothelial cells prevent platelet adhesion and activation by producing potent platelet antagonists, such as the adenosine triphosphate/adenosine diphosphate (ADP) scavenging enzyme CD39/ecto-adenosine diphosphatase,¹⁰ prostacyclin (or prostaglandin I₂ [PGI₂]), and nitric oxide (NO). These molecules also maintain endothelial homeostasis through other mechanisms. NO minimizes leukocyte recruitment to the vessel wall by reducing P-selectin expression on the endothelial surface, decreasing chemokine expression, and reducing transcription of adhesion molecules, such as E-selectin, VCAM-1, and intercellular adhesion molecule-1 (ICAM-1). PGI₂ decreases inflammation by reducing leukocyte adhesion, activation, and extravasation. Additionally, the endothelium supports an extensive repertoire of natural anticoagulant and anti-fibrinolytic pathways, involving glycosaminoglycans (GAGs), thrombomodulin (TM), the activated protein C (APC) pathway, and tissue factor pathway inhibitor (TFPI). The details of these pathways and their roles in regulating microvascular thrombin generation have been described previously.^{10,11}

Under pathological conditions, such as sepsis, IR injury, and disseminated intravascular coagulation (DIC), humoral mediators perturb the homeostatic function of endothelial cells. In sepsis, components of the bacterial cell wall activate pattern recognition receptors (PRRs) on the endothelial surface, leading to cytokine production. Bacterial endotoxin also potently stimulates tissue factor (TF) expression and increased levels of the plasminogen activation inhibitor 1 (PAI-1), blocking fibrinolysis and subsequently resulting in a procoagulant endothelial surface. In IR injury, production of reactive oxygen species is elevated within the microcirculation, leading to a decrease in NO

production and promotion of the adhesive qualities of the endothelial surface. In all cases, endothelial dysfunction is associated with downregulation of key components of the natural anticoagulant system¹¹ (Figure 3).

a-Thrombin: a central mediator of thrombin generation

TF expression within the vasculature is considered a pivotal step in initiating and sustaining coagulation in a broad range of thrombotic diseases.¹² TF is a potent activator of coagulation through its high-affinity binding and activation of factor VII (FVIIa).³ Although primarily produced by cells surrounding the vessel wall, including pericytes and fibroblasts, TF can also be produced intravascularly by endothelial cells, monocytes, and circulating microparticles.^{14,15} Monocytes can synthesize and express TF and are considered a major source of blood-borne TF,¹⁶ with several thrombotic conditions associated with TF expression on circulating monocytes, including chronic inflammation and Gram-negative sepsis.¹⁶ Whether neutrophils and platelets can produce pathophysiologically relevant levels of TF remains controversial.¹⁷

During sepsis, pathogen-associated molecular patterns, expressed by invading bacteria, are recognized by PRRs present on the surface of endothelial cells, platelets, and leukocytes.¹⁸ PRRs transduce signals leading to the release of inflammatory cytokines and chemokines and increased expression of leukocyte-adhesion molecules. They also undermine the natural anticoagulant and fibrinolytic system on endothelial cells¹⁹ and increase TF production by monocytes and endothelial cells.²⁰ The importance of TF in promoting thrombin generation in sepsis has been confirmed using pharmacological TF inhibitors or through the study of mice expressing very low levels of TF, wherein inhibition or low expression of TF in mice exposed to endotoxin attenuates coagulation and inflammation and improves survival.²¹ Importantly, selective deletion of TF from endothelial cells does not decrease a-thrombin generation or improve survival in a preclinical model of endotoxemia,²⁰ suggesting that nonendothelial sources of TF are the dominant trigger of coagulation *in vivo*. In humans, TF inhibition alone does not effectively inhibit a-thrombin generation and inflammation during sepsis, suggesting the existence of alternative pathways.²²

An additional pathway promoting a-thrombin generation is the contact-activated (intrinsic) pathway of coagulation. In this pathway, coagulation cascade activation is initiated by the exposure of negatively charged surfaces, such as the release of RNA or DNA from damaged or dying cells,²³ or secretion of negatively charged inorganic polyphosphates (PolyPs) by platelets.²³ This pathway has been shown to be particularly important in IR injury. a-Thrombin is also a potent activator of platelets, facilitating platelet procoagulant function and fibrin generation through the surface expression of phosphatidylserine.^{24,25} Studies examining a-thrombin generation during cerebral IR injury have identified a crucial role for PolyPs (released by activated platelets) in inducing activation of the contact pathway of coagulation.²⁶ These findings have been corroborated using FXIIa²⁷ and FIXa²⁸ inhibitors, factors involved in the contact pathway of coagulation that appear to reduce

- physiology and pathology of autoimmune disorders. *Rheumatol Int.* 2018;38(6):959-974.
8. Aird WC. Spatial and temporal dynamics of the endothelium. *J Thromb Haemost.* 2005;3(7):1392-1406.
 9. Wolinsky H. A proposal linking clearance of circulating lipoproteins to tissue metabolic activity as a basis for understanding atherogenesis. *Circ Res.* 1980;47(3):301-311.
 10. Jin RC, Voetsch B, Loscalzo J. Endogenous mechanisms of inhibition of platelet function. *Microcirculation.* 2005;12(3):247-258.
 11. Esmon CT. Coagulation inhibitors in inflammation. *Biochem Soc Trans.* 2005;33(Pt 2):401-405.
 12. Erlich JH, Boyle EM, Labriola J, et al. Inhibition of the tissue factor-thrombin pathway limits infarct size after myocardial ischemia-reperfusion injury by reducing inflammation. *Am J Pathol.* 2000;157(6):1849-1862.
 13. Mackman N, Tilley RE, Key NS. Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol.* 2007;27(8):1687-1693.
 14. Levi M, van der Poll T. Coagulation and sepsis. *Thromb Res.* 2017;149:38-44.
 15. Ruf W, Riewald M. Regulation of tissue factor expression. In: *Madame Curie Bioscience Database*. Austin, TX: Landes Bioscience; 2013. <https://www.ncbi.nlm.nih.gov/books/NBK6620/>. Accessed 20 September 2018.
 16. Østerud B, Bjorklid E. Blood-borne tissue factor (including microparticles). In: *Madame Curie Bioscience Database*. Austin, TX: Landes Bioscience; 2013. <https://www.ncbi.nlm.nih.gov/books/NBK6341/>. Accessed 20 September 2018.
 17. Osterud B, Bjorklid E. Tissue factor in blood cells and endothelial cells. *Front Biosci (Elite Ed).* 2012;4(1):289-299.
 18. Raymond SL, Holden DC, Mira JC, et al. Microbial recognition and danger signals in sepsis and trauma. *Biochim Biophys Acta Mol Basis Dis.* 2017;1863(10 Pt B):2564-2573.
 19. Opal SM, Esmon CT. Bench-to-bedside review: functional relationships between coagulation and the innate immune response and their respective roles in the pathogenesis of sepsis. *Crit Care.* 2003;7(1):23-38.
 20. Pawlinski R, Wang JG, Owens AP III, et al. Hematopoietic and nonhematopoietic cell tissue factor activates the coagulation cascade in endotoxemic mice. *Blood.* 2010;116(5):806-814.
 21. Pawlinski R, Pedersen B, Schabbauer G, et al. Role of tissue factor and protease-activated receptors in a mouse model of endotoxemia. *Blood.* 2004;103(4):1342-1347.
 22. Abraham E, Reinhart K, Opal S, et al; OPTIMIST Trial Study Group. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. *JAMA.* 2003;290(2):238-247.
 23. Gajisiewicz JM, Smith SA, Morrissey JH. Polyphosphate and RNA differentially modulate the contact pathway of blood clotting. *J Biol Chem.* 2017;292(5):1808-1814.
 24. Lhermusier T, Chap H, Payrastre B. Platelet membrane phospholipid asymmetry: from the characterization of a scramblase activity to the identification of an essential protein mutated in Scott syndrome. *J Thromb Haemost.* 2011;9(10):1883-1891.
 25. Jackson SP, Schoenwaelder SM. Procoagulant platelets: are they necrotic? *Blood.* 2010;116(12):2011-2018.
 26. Choi SH, Smith SA, Morrissey JH. Polyphosphate is a cofactor for the activation of factor XI by thrombin. *Blood.* 2011;118(26):6963-6970.
 27. Kleinschnitz C, Stoll G, Bendszus M, et al. Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. *J Exp Med.* 2006;203(3):513-518.
 28. Choudhri TF, Hoh BL, Prestigiacomo CJ, et al. Targeted inhibition of intrinsic coagulation limits cerebral injury in stroke without increasing intracerebral hemorrhage. *J Exp Med.* 1999;190(1):91-99.
 29. DeLa Cadena RA, Laskin KJ, Pixley RA, et al. Role of kallikrein-kinin system in pathogenesis of bacterial cell wall-induced inflammation. *Am J Physiol.* 1991;260(2 Pt 1):G213-G219.
 30. Tapper H, Herwald H. Modulation of hemostatic mechanisms in bacterial infectious diseases. *Blood.* 2000;96(7):2329-2337.
 31. Huntington JA. Molecular recognition mechanisms of thrombin. *J Thromb Haemost.* 2005;3(8):1861-1872.
 32. Matafonov A, Sarilla S, Sun MF, et al. Activation of factor XI by products of prothrombin activation. *Blood.* 2011;118(2):437-445.
 33. Coughlin SR. How the protease thrombin talks to cells. *Proc Natl Acad Sci USA.* 1999;96(20):11023-11027.
 34. Adams MN, Ramachandran R, Yau MK, et al. Structure, function and pathophysiology of protease activated receptors. *Pharmacol Ther.* 2011;130(3):248-282.
 35. Camerer E, Kataoka H, Kahn M, Lease K, Coughlin SR. Genetic evidence that protease-activated receptors mediate factor Xa signaling in endothelial cells. *J Biol Chem.* 2002;277(18):16081-16087.
 36. Borensztajn K, Peppelenbosch MP, Spek CA. Factor Xa: at the crossroads between coagulation and signaling in physiology and disease. *Trends Mol Med.* 2008;14(10):429-440.
 37. Furuhashi I, Abe K, Sato T, Inoue H. Thrombin-stimulated proliferation of cultured human synovial fibroblasts through proteolytic activation of proteinase-activated receptor-1. *J Pharmacol Sci.* 2008;108(1):104-111.
 38. Hsieh HL, Tung WH, Wu CY, et al. Thrombin induces EGF receptor expression and cell proliferation via a PKC(delta)/c-Src-dependent pathway in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 2009;29(10):1594-1601.
 39. Kastl SP, Speidl WS, Katsaros KM, et al. Thrombin induces the expression of oncostatin M via AP-1 activation in human macrophages: a link between coagulation and inflammation. *Blood.* 2009;114(13):2812-2818.
 40. Tull SP, Bevins A, Kuravi SJ, et al. PR3 and elastase alter PAR1 signaling and trigger vWF release via a calcium-independent mechanism from glomerular endothelial cells. *PLoS One.* 2012;7(8):e43916.
 41. Okada M, Suzuki K, Takada K, Nakashima M, Nakanishi T, Shinohara T. Detection of up-regulated genes in thrombin-stimulated human umbilical vein endothelial cells. *Thromb Res.* 2006;118(6):715-721.
 42. Fager AM, Wood JP, Bouchard BA, Feng P, Tracy PB. Properties of procoagulant platelets: defining and characterizing the subpopulation binding a functional prothrombinase. *Arterioscler Thromb Vasc Biol.* 2010;30(12):2400-2407.
 43. Lopez E, Bermejo N, Berna-Ero A, et al. Relationship between calcium mobilization and platelet α - and δ -granule secretion. A role for TRPC6 in thrombin-evoked δ -granule exocytosis. *Arch Biochem Biophys.* 2015;585:75-81.
 44. Shankar H, Garcia A, Prabhakar J, Kim S, Kunapuli SP. P2Y12 receptor-mediated potentiation of thrombin-induced thromboxane A2 generation in platelets occurs through regulation of Erk1/2 activation. *J Thromb Haemost.* 2006;4(3):638-647.
 45. Coppinger JA, Cagney G, Toomey S, et al. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood.* 2004;103(6):2096-2104.
 46. Kaplan ZS, Zarpellon A, Alwis I, et al. Thrombin-dependent intravascular leukocyte trafficking regulated by fibrin and the platelet receptors GPIb and PAR4. *Nat Commun.* 2015;6(1):7835.
 47. Martinez JT, Fernandez G, Vazquez-Leon H. Clinical evaluation of new therapeutic concepts in septic shock. *Obstet Gynecol.* 1966;27(2):296-301.
 48. Jaimes F, De La Rosa G, Morales C, et al. Unfractionated heparin for treatment of sepsis: a randomized clinical trial (The HETRASE Study). *Crit Care Med.* 2009;37(4):1185-1196.
 49. Umemura Y, Yamakawa K, Ogura H, Yuhara H, Fujimi S. Efficacy and safety of anticoagulant therapy in three specific populations with sepsis: a meta-analysis of randomized controlled trials. *J Thromb Haemost.* 2016;14(3):518-530.
 50. Zarychanski R, Abou-Setta AM, Kanji S, et al; Canadian Critical Care Trials Group. The efficacy and safety of heparin in patients with sepsis: a systematic review and meta-analysis. *Crit Care Med.* 2015;43(3):511-518.
 51. Fan Y, Jiang M, Gong D, Zou C. Efficacy and safety of low-molecular-weight heparin in

- patients with sepsis: a meta-analysis of randomized controlled trials. *Sci Rep*. 2016;6(1):25984.
52. Rhodes A, Evans LE, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. *Intensive Care Med*. 2017;43(3):304-377.
 53. Duensing TD, Wing JS, van Putten JP. Sulfated polysaccharide-directed recruitment of mammalian host proteins: a novel strategy in microbial pathogenesis. *Infect Immun*. 1999;67(9):4463-4468.
 54. Fourrier F, Jourdain M, Tournays A. Clinical trial results with antithrombin III in sepsis. *Crit Care Med*. 2000;28(9 suppl):S38-S43.
 55. Allen KS, Sawheny E, Kinasewitz GT. Anticoagulant modulation of inflammation in severe sepsis. *World J Crit Care Med*. 2015;4(2):105-115.
 56. Allingstrup M, Wetterslev J, Ravn FB, Møller AM, Afshari A. Antithrombin III for critically ill patients: a systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med*. 2016;42(4):505-520.
 57. Griffin JH, Zlokovic BV, Mosnier LO. Activated protein C: biased for translation. *Blood*. 2015;125(19):2898-2907.
 58. Bernard GR, Vincent JL, Laterre PF, et al; Recombinant human protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med*. 2001;344(10):699-709.
 59. Kylat RI, Ohlsson A. Recombinant human activated protein C for severe sepsis in neonates. *Cochrane Database Syst Rev*. 2012; (4):CD005385.
 60. Guo H, Singh I, Wang Y, et al. Neuroprotective activities of activated protein C mutant with reduced anticoagulant activity. *Eur J Neurosci*. 2009;29(6):1119-1130.
 61. Mosnier LO, Gale AJ, Yegneswaran S, Griffin JH. Activated protein C variants with normal cytoprotective but reduced anticoagulant activity. *Blood*. 2004;104(6):1740-1744.
 62. Covic L, Misra M, Badar J, Singh C, Kuliopulos A. Pepsin-based intervention of thrombin-receptor signaling and systemic platelet activation. *Nat Med*. 2002;8(10):1161-1165.
 63. Slofstra SH, Bijlsma MF, Groot AP, et al. Protease-activated receptor-4 inhibition protects from multiorgan failure in a murine model of systemic inflammation. *Blood*. 2007;110(9):3176-3182.
 64. Aisiku O, Peters CG, De Ceunynck K, et al. Parmodulins inhibit thrombus formation without inducing endothelial injury caused by vorapaxar. *Blood*. 2015;125(12):1976-1985.
 65. French SL, Arthur JF, Tran HA, Hamilton JR. Approval of the first protease-activated receptor antagonist: rationale, development, significance, and considerations of a novel anti-platelet agent. *Blood Rev*. 2015;29(3):179-189.
 66. Mao Y, Zhang M, Tuma RF, Kunapuli SP. Deficiency of PAR4 attenuates cerebral ischemia/reperfusion injury in mice. *J Cereb Blood Flow Metab*. 2010;30(5):1044-1052.
 67. Routhu KV, Tsopanoglou NE, Strande JL. Parstatin(1-26): the putative signal peptide of protease-activated receptor 1 confers potent protection from myocardial ischemia-reperfusion injury. *J Pharmacol Exp Ther*. 2010;332(3):898-905.
 68. Yamakawa K, Aihara M, Ogura H, Yuhara H, Hamasaki T, Shimazu T. Recombinant human soluble thrombomodulin in severe sepsis: a systematic review and meta-analysis. *J Thromb Haemost*. 2015;13(4):508-519.
 69. Ito T, Kakhana Y, Maruyama I. Thrombomodulin as an intravascular safeguard against inflammatory and thrombotic diseases. *Expert Opin Ther Targets*. 2016;20(2):151-158.
 70. Saito H, Maruyama I, Shimazaki S, et al. Efficacy and safety of recombinant human soluble thrombomodulin (ART-123) in disseminated intravascular coagulation: results of a phase III, randomized, double-blind clinical trial. *J Thromb Haemost*. 2007;5(1):31-41.
 71. Vincent JL, Ramesh MK, Ernest D, et al. A randomized, double-blind, placebo-controlled, phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. *Crit Care Med*. 2013;41(9):2069-2079.
 72. Goldfarb RD, Glock D, Johnson K, et al. Randomized, blinded, placebo-controlled trial of tissue factor pathway inhibitor in porcine septic shock. *Shock*. 1998;10(4):258-264.
 73. Matyal R, Vin Y, Delude RL, Lee C, Creasey AA, Fink MP. Extremely low doses of tissue factor pathway inhibitor decrease mortality in a rabbit model of septic shock. *Intensive Care Med*. 2001;27(8):1274-1280.
 74. Creasey AA, Chang AC, Feigen L, Wün TC, Taylor FB Jr, Hinshaw LB. Tissue factor pathway inhibitor reduces mortality from *Escherichia coli* septic shock. *J Clin Invest*. 1993;91(6):2850-2860.
 75. de Jonge E, Dekkers PE, Creasey AA, et al. Tissue factor pathway inhibitor dose-dependently inhibits coagulation activation without influencing the fibrinolytic and cytokine response during human endotoxemia. *Blood*. 2000;95(4):1124-1129.
 76. Abraham E, Reinhart K, Svoboda P, et al. Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with severe sepsis: a multicenter, randomized, placebo-controlled, single-blind, dose escalation study. *Crit Care Med*. 2001;29(11):2081-2089.
 77. Renné T, Nieswandt B, Gailani D. The intrinsic pathway of coagulation is essential for thrombus stability in mice. *Blood Cells Mol Dis*. 2006;36(2):148-151.
 78. Müller F, Gailani D, Renné T. Factor XI and XII as antithrombotic targets. *Curr Opin Hematol*. 2011;18(5):349-355.
 79. Salomon O, Steinberg DM, Zucker M, Varon D, Zivelin A, Seligsohn U. Patients with severe factor XI deficiency have a reduced incidence of deep-vein thrombosis. *Thromb Haemost*. 2011;105(2):269-273.
 80. Salomon O, Steinberg DM, Koren-Morag N, Tanne D, Seligsohn U. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. *Blood*. 2008;111(8):4113-4117.
 81. Renné T. The procoagulant and proinflammatory plasma contact system. *Semin Immunopathol*. 2012;34(1):31-41.
 82. Kenne E, Renné T. Factor XII: a drug target for safe interference with thrombosis and inflammation. *Drug Discov Today*. 2014;19(9):1459-1464.
 83. Larsson M, Rayzman V, Nolte MW, et al. A factor XIIa inhibitory antibody provides thromboprotection in extracorporeal circulation without increasing bleeding risk. *Sci Transl Med*. 2014;6(222):222ra17.
 84. Heydenreich N, Nolte MW, Göb E, et al. C1-inhibitor protects from brain ischemia-reperfusion injury by combined anti-inflammatory and antithrombotic mechanisms. *Stroke*. 2012;43(9):2457-2467.
 85. Krupka J, May F, Weimer T, et al. The coagulation factor XIIa inhibitor rHA-infestin-4 improves outcome after cerebral ischemia/reperfusion injury in rats. *PLoS One*. 2016;11(1):e0146783.
 86. Gailani D, Bane CE, Gruber A. Factor XI and contact activation as targets for antithrombotic therapy. *J Thromb Haemost*. 2015;13(8):1383-1395.
 87. Tucker EI, Verbout NG, Leung PY, et al. Inhibition of factor XI activation attenuates inflammation and coagulopathy while improving the survival of mouse polymicrobial sepsis. *Blood*. 2012;119(20):4762-4768.
 88. Chen W, Carvalho LP, Chan MY, Kini RM, Kang TS. Fasxiator, a novel factor XIa inhibitor from snake venom, and its site-specific mutagenesis to improve potency and selectivity. *J Thromb Haemost*. 2015;13(2):248-261.
 89. Büller HR, Bethune C, Bhanot S, et al; FXI-ASO TKA Investigators. Factor XI anti-sense oligonucleotide for prevention of venous thrombosis. *N Engl J Med*. 2015;372(3):232-240.
 90. Morrissey JH, Smith SA. Polyphosphate as modulator of hemostasis, thrombosis, and inflammation. *J Thromb Haemost*. 2015;13(suppl 1):S92-S97.
 91. Travers RJ, Shenoi RA, Kalathottukaren MT, Kizhakkedathu JN, Morrissey JH. Nontoxic polyphosphate inhibitors reduce thrombosis while sparing hemostasis. *Blood*. 2014;124(22):3183-3190.
 92. Slaba I, Wang J, Kolaczowska E, McDonald B, Lee WY, Kubes P. Imaging the dynamic platelet-neutrophil response in sterile liver injury and repair in mice. *Hepatology*. 2015;62(5):1593-1605.
 93. Akinosoglou K, Alexopoulos D. Use of anti-platelet agents in sepsis: a glimpse into the future. *Thromb Res*. 2014;133(2):131-138.

94. Hinshaw LB, Solomon LA, Erdös EG, Reins DA, Gunter BJ. Effects of acetylsalicylic acid on the canine response to endotoxin. *J Pharmacol Exp Ther.* 1967;157(3):665-671.
95. Halushka PV, Wise WC, Cook JA. Protective effects of aspirin in endotoxic shock. *J Pharmacol Exp Ther.* 1981;218(2):464-469.
96. Blasco-Colmenares E, Perl TM, Guallar E, et al. Aspirin plus clopidogrel and risk of infection after coronary artery bypass surgery. *Arch Intern Med.* 2009;169(8):788-796.
97. Bonaca MP, Goto S, Bhatt DL, et al. Prevention of stroke with ticagrelor in patients with prior myocardial infarction: insights from PEGASUS-TIMI 54 (Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin-Thrombolysis in Myocardial Infarction 54). *Circulation.* 2016;134(12):861-871.
98. Otto GP, Sossdorf M, Boettel J, et al. Effects of low-dose acetylsalicylic acid and atherosclerotic vascular diseases on the outcome in patients with severe sepsis or septic shock. *Platelets.* 2013;24(6):480-485.
99. Wang X, Zhao X, Johnston SC, et al; CHANCE investigators. Effect of clopidogrel with aspirin on functional outcome in TIA or minor stroke: CHANCE substudy. *Neurology.* 2015;85(7):573-579.
100. Gross AK, Dunn SP, Feola DJ, et al. Clopidogrel treatment and the incidence and severity of community acquired pneumonia in a cohort study and meta-analysis of antiplatelet therapy in pneumonia and critical illness. *J Thromb Thrombolysis.* 2013; 35(2):147-154.
101. Walther A, Czabanka M, Gebhard MM, Martin E. Glycoprotein IIb/IIIa-inhibition and microcirculatory alterations during experimental endotoxemia—an intravital microscopic study in the rat. *Microcirculation.* 2004;11(1):79-88.
102. Taylor FB, Collier BS, Chang AC, et al. 7E3 F(ab)₂, a monoclonal antibody to the platelet GPIIb/IIIa receptor, protects against microangiopathic hemolytic anemia and microvascular thrombotic renal failure in baboons treated with C4b binding protein and a sublethal infusion of *Escherichia coli*. *Blood.* 1997;89(11):4078-4084.
103. Pu Q, Wiel E, Corseau D, et al. Beneficial effect of glycoprotein IIb/IIIa inhibitor (AZ-1) on endothelium in *Escherichia coli* endotoxin-induced shock. *Crit Care Med.* 2001;29(6):1181-1188.
104. Sharron M, Hoptay CE, Wiles AA, et al. Platelets induce apoptosis during sepsis in a contact-dependent manner that is inhibited by GPIIb/IIIa blockade. *PLoS One.* 2012;7(7):e41549.
105. Liverani E, Rico MC, Tsygankov AY, Kilpatrick LE, Kunapuli SP. P2Y₁₂ receptor modulates sepsis-induced inflammation. *Arterioscler Thromb Vasc Biol.* 2016;36(5):961-971.
106. Sexton TR, Zhang G, Macaulay TE, et al. Ticagrelor reduces thromboinflammatory markers in patients with pneumonia. *JACC Basic Transl Sci.* 2018;3(4):435-449.
107. Furman MI, Krueger LA, Linden MD, et al. GPIIb-IIIa antagonists reduce thromboinflammatory processes in patients with acute coronary syndromes undergoing percutaneous coronary intervention. *J Thromb Haemost.* 2005;3(2):312-320.
108. Kingma JG Jr, Plante S, Bogaty P. Platelet GPIIb/IIIa receptor blockade reduces infarct size in a canine model of ischemia-reperfusion. *J Am Coll Cardiol.* 2000;36(7):2317-2324.
109. Kupatt C, Habazettl H, Hanusch P, et al. c7E3Fab reduces postischemic leukocyte-thrombocyte interaction mediated by fibrinogen. Implications for myocardial reperfusion injury. *Arterioscler Thromb Vasc Biol.* 2000;20(10):2226-2232.
110. Maeda M, Moriguchi A, Mihara K, et al. FK419, a nonpeptide platelet glycoprotein IIb/IIIa antagonist, ameliorates brain infarction associated with thrombotic focal cerebral ischemia in monkeys: comparison with tissue plasminogen activator. *J Cereb Blood Flow Metab.* 2005;25(1):108-118.
111. Hu H, Batteux F, Chéreau C, et al. Clopidogrel protects from cell apoptosis and oxidative damage in a mouse model of renal ischaemia-reperfusion injury. *J Pathol.* 2011; 225(2):265-275.
112. Serebruany VL, Malinin AI, Eisert RM, Sane DC. Risk of bleeding complications with antiplatelet agents: meta-analysis of 338,191 patients enrolled in 50 randomized controlled trials. *Am J Hematol.* 2004;75(1):40-47.
113. Sørensen R, Hansen ML, Abildstrom SZ, et al. Risk of bleeding in patients with acute myocardial infarction treated with different combinations of aspirin, clopidogrel, and vitamin K antagonists in Denmark: a retrospective analysis of nationwide registry data. *Lancet.* 2009;374(9706):1967-1974.
114. Goebel S, Li Z, Vogelmann J, et al. The GPVI-Fc fusion protein revacept improves cerebral infarct volume and functional outcome in stroke. *PLoS One.* 2013;8(7):e66960.
115. Ungerer M, Rosport K, Bültmann A, et al. Novel antiplatelet drug revacept (dimeric glycoprotein VI-Fc) specifically and efficiently inhibited collagen-induced platelet aggregation without affecting general hemostasis in humans. *Circulation.* 2011;123(17):1891-1899.
116. Kraft P, Schuhmann MK, Fluri F, et al. Efficacy and safety of platelet glycoprotein receptor blockade in aged and comorbid mice with acute experimental stroke. *Stroke.* 2015; 46(12):3502-3506.
117. Claushuis TAM, de Vos AF, Nieswandt B, et al. Platelet glycoprotein VI aids in local immunity during pneumonia-derived sepsis caused by gram-negative bacteria. *Blood.* 2018;131(8):864-876.
118. Hitchcock JR, Cook CN, Bobat S, et al. Inflammation drives thrombosis after *Salmonella* infection via CLEC-2 on platelets. *J Clin Invest.* 2015;125(12):4429-4446.
119. Rayes J, Lax S, Wichaiyo S, et al. The podoplanin-CLEC-2 axis inhibits inflammation in sepsis. *Nat Commun.* 2017; 8(1):2239.
120. Washington AV, Schubert RL, Quigley L, et al. A TREM family member, TLT-1, is found exclusively in the alpha-granules of megakaryocytes and platelets. *Blood.* 2004;104(4):1042-1047.
121. Derive M, Bouazza Y, Sennoun N, et al. Soluble TREM-like transcript-1 regulates leukocyte activation and controls microbial sepsis. *J Immunol.* 2012;188(11):5585-5592.
122. Washington AV, Gibot S, Acevedo I, et al. TREM-like transcript-1 protects against inflammation-associated hemorrhage by facilitating platelet aggregation in mice and humans. *J Clin Invest.* 2009;119(6):1489-1501.
123. Morales-Ortiz J, Deal V, Reyes F, et al. Platelet-derived TLT-1 is a prognostic indicator in ALI/ARDS and prevents tissue damage in the lungs in a mouse model. *Blood.* 2018;132(23):2495-2505.
124. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev.* 2007;21(2):99-111.
125. Ott I, Neumann FJ, Gawaz M, Schmitt M, Schömig A. Increased neutrophil-platelet adhesion in patients with unstable angina. *Circulation.* 1996;94(6):1239-1246.
126. Zarbock A, Ley K. The role of platelets in acute lung injury (ALI). *Front Biosci. (Landmark Ed).* 2009;14:150-158.
127. Schmitt C, Abt M, Ciorciaro C, et al. First-in-man study with inclacumab, a human monoclonal antibody against P-selectin. *J Cardiovasc Pharmacol.* 2015;65(6):611-619.
128. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood.* 2003;101(10):3765-3777.
129. Sakr Y, Dubois M-J, De Backer D, Creteur J, Vincent J-L. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med.* 2004;32(9):1825-1831.
130. Zeni F, Freeman B, Natanson C. Anti-inflammatory therapies to treat sepsis and septic shock: a reassessment. *Crit Care Med.* 1997;25(7):1095-1100.
131. Galasso G, Schiekofer S, D'Anna C, et al. No-reflow phenomenon: pathophysiology, diagnosis, prevention, and treatment. A review of the current literature and future perspectives. *Angiology.* 2014;65(3):180-189.
132. Bouton MC, Boulaftali Y, Richard B, Arocas V, Michel JB, Jandrot-Perrus M. Emerging role of serpinE2/protease nexin-1 in hemostasis and vascular biology. *Blood.* 2012;119(11):2452-2457.
133. Petrey AC, de la Motte CA. Thrombin cleavage of inter-alpha-inhibitor heavy chain 1 regulates leukocyte binding to an inflammatory hyaluronan matrix. *J Biol Chem.* 2016;291(47):24324-24334.
134. Huber-Lang M, Sarma JV, Zetoune FS, et al. Generation of C5a in the absence of C3: a new complement activation pathway. *Nat Med.* 2006;12(6):682-687.
135. Krisinger MJ, Goebeler V, Lu Z, et al. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. *Blood.* 2012;120(8):1717-1725.