Roles of Coagulation Proteases and PARs (Protease-Activated Receptors) in Mouse Models of Inflammatory Diseases

Jens J. Posma, Steven P. Grover, Yohei Hisada, A. Phillip Owens III, Silvio Antoniak, Henri M. Spronk, Nigel Mackman

Abstract—Activation of the blood coagulation cascade leads to fibrin deposition and platelet activation that are required for hemostasis. However, aberrant activation of coagulation can lead to thrombosis. Thrombi can cause tissue ischemia, and fibrin degradation products and activated platelets can enhance inflammation. In addition, coagulation proteases activate cells by cleavage of PARs (protease-activated receptors), including PAR1 and PAR2. Direct oral anticoagulants have recently been developed to specifically inhibit the coagulation proteases FXa (factor Xa) and thrombin. Administration of these inhibitors to wild-type mice can be used to determine the roles of FXa and thrombin in different inflammatory diseases. These results can be compared with the phenotypes of mice with deficiencies of either Par1 (F2r) or Par2 (F2rl1). However, inhibition of coagulation proteases will have effects beyond reducing PAR signaling, and a deficiency of PARs will abolish signaling from all proteases that activate these receptors. We will summarize studies that examine the roles of coagulation proteases, particularly FXa and thrombin, and PARs in different mouse models of inflammatory disease. Targeting FXa and thrombin or PARs may reduce inflammatory diseases in humans.

Key Words: anticoagulants ■ blood coagulation ■ inflammation ■ models, animal ■ thrombin

The primary function of the blood coagulation cascade is to prevent blood loss after vessel injury. This cascade is initiated by the TF (tissue factor)/FVIIa (factor VIIa) complex. FXa (factor Xa) and thrombin are components of the so-called common pathway.[1–3] Thrombin is the central protease of the blood coagulation cascade and has numerous roles, including cleavage of fibrinogen to fibrin and activation of platelets (Figure). Aberrant activation of the blood coagulation system can contribute to the pathology of various diseases. Formation of thrombi within blood vessels leads to ischemia and inflammation. In addition, fibrin degradation products are released during the degradation of fibrin, that can enhance inflammation. For instance, the E1 fragment increases the recruitment of leukocytes to sites of injury by binding to VE-cadherin on endothelial cells and integrin αMβ2 on leukocytes.[4] In addition, macrophage activation is enhanced by binding to fibrin(ogen) via αMβ2.[5] Moreover, activated platelets can enhance inflammation by releasing inflammatory mediators and by promoting extravasation of leukocytes.[6] Finally, FXa and thrombin activate a variety of cell types, including endothelial cells, vascular smooth muscle cells, macrophages, fibroblasts, and cardiac myocytes, via PARs (protease-activated receptor; Figure). There are 4 members of the PAR family (PAR1–4), and they are ubiquitously expressed. These receptors belong to the large family of G-protein–coupled receptors but are unique because they are activated by proteolytic cleavage that exposes a tethered ligand. Thrombin activates PAR1, PAR3, and PAR4, whereas FXa primarily activates PAR2.[7–10] Importantly, other proteases also activate PAR1 and PAR2. For example, APC (activated protein C) and matrix metalloproteases activate PAR1,[11–13] whereas trypsins, tryptase, FVIIa, and matriptase activate PAR2.[14–16] In addition, PAR1 and PAR2 can form heterodimers[17,18] Interestingly, human platelets express PAR1 and PAR4, whereas mouse platelets express a PAR3/PAR4 complex.[19–21] This means that any phenotype observed in *Par1*$^{-/-}$ mice cannot be because of an attenuation of platelet activation. Furthermore, there may be differences in PAR signaling in mice and humans.

A number of anticoagulants, such as vitamin K antagonists and various forms of heparins, are used to prevent and treat venous thrombosis and prevent stroke associated with atrial fibrillation.[22] Direct oral anticoagulants (DOACs), which are also known as non-vitamin K antagonist oral anticoagulants, are a relatively new class of anticoagulants that specifically

Received on: August 23, 2018; final version accepted on: October 19, 2018.

From the Laboratory for Clinical Thrombosis and Hemostasis (J.J.P., H.M.S.), Department of Internal Medicine (J.J.P., H.M.S.), and Department of Biochemistry (J.J.P., H.M.S.), Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, The Netherlands; Thrombosis and Hemostasis Program, Division of Hematology and Oncology, Department of Medicine (S.P.G., Y.H., N.M.) and Department of Pathology and Laboratory Medicine (S.A.), University of North Carolina, Chapel Hill; and Division of Cardiovascular Health and Disease, University of Cincinnati College of Medicine, OH (A.P.O.).

Correspondence to Nigel Mackman, PhD, Thrombosis and Hemostasis Program, Department of Medicine, University of North Carolina at Chapel Hill, 111 Mason Farm Rd, 2312C Medical Biomolecular Research Bldg, CB 7126, Chapel Hill, NC 27599. Email nmackman@med.unc.edu

Nonstandard Abbreviations and Acronyms

APC	activated protein C
CVB3	coxsackievirus B3
DOAC	direct oral anticoagulant
FVIIa	factor VIIa
FXa	factor Xa
HFD	high-fat diet
I/R	ischemia-reperfusion
I/R-O	ischemia-reperfusion open chest
IAV	influenza A virus
LAD	left anterior descending
PAR	protease-activated receptor
TF	tissue factor
TLR3	Toll-like receptor 3

inhibit FXa and thrombin.[23,24] There are several FXa inhibitors (apixaban, betrixaban, edoxaban, and rivaroxaban) but only 1 approved thrombin inhibitor called dabigatran etexilate, which is a prodrug that is metabolized to dabigatran.[24] Various DOACs have been used to study the roles of FXa and thrombin in various mouse models of disease, but rivaroxaban and dabigatran are the most popular. As expected, therapeutic doses of these inhibitors reduce thrombosis in mice.[25–27] We will not discuss studies that investigated the antithrombotic activities of these drugs. One would expect different effects of rivaroxaban versus dabigatran on PAR signaling. For instance, inhibiting FXa would be expected to primarily inhibit PAR2 signaling, whereas inhibiting thrombin would primarily inhibit PAR1 signaling, as well as PAR3 and PAR4 signaling (Figure). Additionally, *Par2*$^{-/-}$ and *Par1*$^{-/-}$ mice can be used to compare and contrast the effects seen in wild-type mice with pharmacological inhibition of either FXa or thrombin, respectively. However, inhibition of proteases will have effects beyond PAR signaling, and a *Par* deficiency will abolish signaling from multiple proteases. In addition, studies with *Par1*$^{-/-}$ or *Par2*$^{-/-}$ mice do not provide any information on the protease(s) that are activating the PARs in a given disease

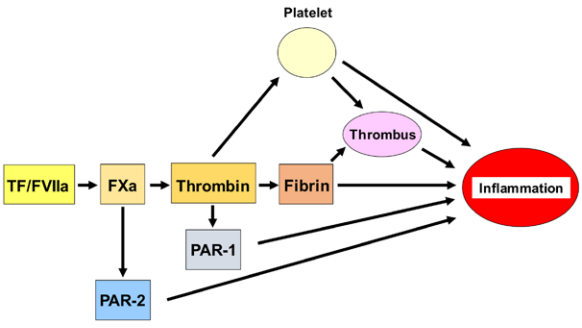

Figure. Roles of coagulation proteases and downstream pathways in inflammatory diseases. Activation of the coagulation cascade leads to cleavage of fibrinogen into fibrin and platelet activation that can contribute to thrombosis. Platelet activation, and fibrin degradation products can also enhance inflammation. Additionally, coagulation proteases can activate cells via various PARs (protease-activated receptors) that can increase the expression of inflammatory mediators. FVIIa indicates factor VIIa; FXa, factor Xa; and TF, tissue factor.

or the effector cells that are expressing the PARs. Recently, transgenic mouse lines containing floxed *Par1* and *Par2* genes have been generated (J. Palumbo, unpublished data, 2018; E. Camerer, unpublished data, 2018), and these mice are being used to determine the role of PAR1 and PAR2 in different cell types in multiple diseases. Similarly, transgenic mice have been made that express mutant forms of PAR1 and PAR2 that cannot be activated by particular proteases. For instance, PAR1^{R41Q} cannot be activated by either thrombin or APC, whereas PAR1^{R46Q} cannot be activated by APC.[28] Another mouse line was generated that expresses PAR2^{G37I} that is resistant to activation by FXa.[29] These mice will be useful to determine the role of different proteases in the activation of PARs in various diseases.

In this review, we summarize the effects of inhibiting different coagulation proteases, particularly FXa and thrombin, in wild-type mice and compare these results with the phenotypes of *Par1*- and *Par2*-deficient mice in various mouse models of inflammatory disease. We will not discuss studies on cancer because this topic has been reviewed previously.[30–33]

Doses of Rivaroxaban and Dabigatran Etexilate Used in Mouse Studies

The therapeutic dose of rivaroxaban used in humans is between 0.13 and 0.27 mg/kg (based on a patient weight of 75 kg), which gives peak plasma concentrations of 122 to 250 ng/mL.[34,35] Dabigatran etexilate has a low bioavailability, and, therefore, it requires a high therapeutic dose in humans between 2 and 4 mg/kg, which gives peak plasma concentrations of 121.6 to 172.9 ng/mL (dose between 2.9 and 4.0 mg/kg).[36–38] When using DOACs in mice, one has to consider the biological differences between humans and mice, including the faster metabolism of mice, the shorter half-life in mice, and the fact that the drugs were developed to inhibit the human coagulation proteases.[39] Therefore, higher doses of these drugs are needed for mice.

Rivaroxaban and dabigatran etexilate as well as other DOACs, such as edoxaban, are administrated to mice via either chow, oral gavage, or intravenous injection (Tables 1 and 2). Most studies report the dose in terms of milligrams per gram of chow. Only some studies report the plasma levels of the drug and present data from plasma-based functional assays that indicate the level of anticoagulation (Tables 1 and 2). Plasma levels of rivaroxaban and dabigatran can be measured using an anti–FXa-based assay and chromatography mass spectrometry, respectively.[40,41]

There is a wide range of doses of rivaroxaban that have been used in mice (0.006–1.2 mg/g chow; Table 1). We found that a rivaroxaban dose of 1.2 mg per chow produced a plasma level of 150 to 260 ng/mL in *apoE*$^{-/-}$ mice fed a Western diet (40.5% fat and 0.25% cholesterol; J. Posma and H. Spronk, unpublished data, 2018). Rivaroxaban induces a linear, concentration-dependent prolongation of the prothrombin time, making it a suitable test to measure the level of anticoagulation with rivaroxaban.[34] One study used 3 different doses of rivaroxaban (0.01, 0.2, and 0.4 mg/g chow) and found a significant increase in prothrombin time with the 2 higher doses.[45] A smaller range of dabigatran etexilate

Table 1. Effects of Rivaroxaban in Mouse Models of Inflammatory Disease

Animal Model	Chow, mg/g	[Rivaroxaban] Plasma, ng/mL	PT, s; Control vs Rivaroxaban	Major Findings	Reference
apoE$^{-/-}$	0.006	6.5		Inflammation↓, stability→	42
apoE$^{-/-}$	0.031	24.2		Inflammation↓, plaque stability↑	42
apoE$^{-/-}$ WD	0.031	28.5		Inflammation↓, atherogenesis↓, plaque stability↑	43
apoE$^{-/-}$ WD	1.2	210		Inflammation↓, atherogenesis↓, plaque stability↑, plaque regression	Unpublished*
Ldlr$^{-/-}$ WD	1.2			Atherogenesis↓	Unpublished
MI I/R-O	0.54	340		Inflammation↓, ejection fraction↑	44
MI I/R-O	1.2	790		Inflammation↓, ejection fraction↑, survival↑	44
MI I/R-C	1.6 mg/kg bodyweight IV			Infarct size↓, ejection fraction↑	Unpublished
MI LAD	0.5	500	10→12	Ejection fraction↑, remodeling↓	40
SCD	0.013	ND	No change		45
SCD	0.2	75	14→22		45
SCD	0.4	190	14→28	Inflammation↓	45
CVB3	0.5			Virus↓	Unpublished
IAV	0.5			Survival↑	Unpublished

apoE$^{-/-}$ indicates apolipoprotein E deficient; CVB3, coxsackievirus B3; I/R-C, ischemia reperfusion closed chest; I/R-O, ischemia-reperfusion open chest; IAV, influenza A virus; LAD, left anterior descending; Ldlr$^{-/-}$, low-density lipoprotein receptor deficient; MI, myocardial infarction; ND, not determined; PT, prothrombin time; SCD, sickle cell disease; TAT, thrombin-antithrombin; and WD, Western diet.

*Thrombin generation assay lag time: 2.2→3.4 min.

doses have been used in different mouse studies (Table 2). Interestingly, the level of absorption of dabigatran etexilate is affected by the type of diet.[46,48] The activated partial thromboplastin time correlates with the level of dabigatran at therapeutic plasma levels and is used to monitor the anticoagulation of this DOAC. However, the activated partial thromboplastin time loses sensitivity at supratherapeutic levels of dabigatran (>200 ng/mL).[54,55] Thrombin time is sensitive to the levels of dabigatran etexilate at plasma levels <60ng/mL, but no correlation is observed at higher levels.[56]

Table 2. Effects of Dabigatran in Mouse Models of Inflammatory Disease

Animal Model	Chow, mg/g	[Dabigatran] Plasma, ng/mL	aPTT (s) Control vs Dabigatran	Major Findings	Reference
apoE$^{-/-}$ WD	5.6	622		Atherogenesis↓, plaque stability↑	46
apoE$^{-/-}$ WD	7.5	100–200		Atherogenesis↓, plaque stability↑	47
apoE$^{-/-}$ WD	10	372		Atherogenesis↓	48*
apoE$^{-/-}$	10			Atherogenesis↓, plaque stability↑	49
apoE$^{-/-}$; Tm$^{pro/pro}$; cuff WD	7.5			Inflammation↓, atherogenesis↓, plaque stability↑	50†
SCD	0.75	50			45
SCD	5	200			45
SCD	10	190	25→75		45‡
SCD	15	900	25→95	Spontaneous bleeding	Unpublished
NAFLD HFD	10			Inflammation↓, hepatic fibrin↓, steatosis↓	51,52
CVB3	10		25→68	Myocarditis↑	53

apoE$^{-/-}$ indicates apolipoprotein E deficient; aPTT, activated partial thromboplastin time; CVB3, coxsackievirus B3; HFD, high-fat diet; NAFLD, non-alcoholic fatty liver disease; SCD, sickle cell disease; TAT, thrombin-antithrombin; TT, thrombin time; and WD, Western diet.

*TT: 30→93.

†TAT: 546→10 ng/mL.

‡TAT: 14→6 ng/L.

One study evaluated 3 doses of dabigatran (5, 10, and 15 mg/g chow) in mice and found that the 10- and 15-mg/g doses significantly increased the activated partial thromboplastin time.[45] Another study found that a dose of 10 mg/g of dabigatran etexilate increased the thrombin time from 30 to 93 seconds.[48] An oral dose of 100 mg/kg body weight of dabigatran etexilate did not cause any bleeding complication.[57] This study showed that dabigatran is rapidly cleared in mice with a half-life of 1.25 hours. However, sickle cell mice bled spontaneously when dabigatran etexilate was given by chow at a dose of 15 mg/g chow, and wild-type mice bled spontaneously when they received a dose of 500 mg/kg of body weight by oral gavage (E. Sparkenbaugh and R. Pawlinski, unpublished data, 2018).

Atherosclerosis

High levels of TF expression and procoagulant activity are present in human atherosclerotic plaques.[58–60] TF expression has also been observed in a rabbit model of atherosclerosis.[61] In addition, levels of TF expression are increased with progression of human lesions.[62]

A*poE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice are the most common models used to study atherosclerosis in mice.[63] Humans carry the majority of their lipoproteins in the LDL (low-density lipoprotein) subfraction, whereas mice transport their lipoproteins via high-density lipoprotein. Feeding *apoE*$^{-/-}$ and *Ldlr*$^{-/-}$ a Western diet, which is classically regarded as 42% fat and 0.2% cholesterol, results in high levels of LDL that resembles distinct genetic disorders in humans (familial dysbetalipoproteinemia and hypercholesterolemia). However, mice fed a Western diet also have abnormally high chylomicrons and LDL compared with humans. *ApoE*$^{-/-}$ mice develop spontaneous atherosclerotic lesions on regular chow, but atherosclerosis is markedly accelerated by feeding a Western diet.[64,65] In contrast, most studies with *Ldlr*$^{-/-}$ mice use a Western diet because there is limited atherosclerosis on regular chow with young mice. Atherosclerosis is normally measured in the aortic sinus, ascending aorta, and innominate artery. Addition of a cuff around the carotid artery creates vascular shear stress and vascular injury that accelerates atherosclerosis. Deletion of the *apoE* gene interferes with various processes, including macrophage and adipose tissue biology, whereas deletion of the *Ldlr* gene primarily affects LDL clearance from the liver.[66,67] When interpreting mouse data, one also has to consider the differences between mice and humans, including differences in lesion distribution, medial layer size, and lipoprotein transport.[63,68] Both *apoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice fed a Western diet have a prothrombotic state, as measured by increased levels of plasma thrombin-antithrombin complex.[69,70]

Effect of Deficiencies of Procoagulant or Anticoagulant Proteins on Atherosclerosis in Mice

Components of the coagulation system have been implicated in the development of atherosclerosis.[69] Increased atherosclerotic lesion development was observed in *apoE*$^{-/-}$ mice expressing the hypercoagulable Factor V Leiden variant, which is likely because of an increased capacity for thrombin generation.[70] Similarly, a 50% reduction in TF pathway inhibitor—the endogenous inhibitor of TF—increased the development of lesions in *apoE*$^{-/-}$ mice fed regular chow.[71] Conversely, a 50% reduction of TF did not alter atherosclerotic lesion development in *apoE*$^{-/-}$ mice fed regular chow.[72] Similarly, no difference in atherosclerotic lesion development was observed in *Ldlr*$^{-/-}$ mice reconstituted with bone marrow expressing low levels of TF on a Western diet.[72]

Genetic studies have investigated the role of thrombin on the development of atherosclerosis. Interestingly, *apoE*$^{-/-}$ mice with 50% levels of prothrombin had reduced lesion burden on regular chow.[50] The role for fibrinogen—the major physiological substrate of thrombin—in atherosclerosis is model dependent. In addition, it should be noted that *fib*$^{-/-}$ mice are difficult to breed. In the *apoE*$^{-/-}$ model, fibrinogen deficiency did not alter atherosclerotic lesion development in mice on normal chow.[73] However, in a transgenic mouse model expressing human apo(a), deletion of fibrinogen significantly reduced lesion development in mice fed a Western diet because of reduced binding of apo(a) to fibrin(ogen) in the vessel wall.[74] Finally, fibrinogen deficiency increased plaque development in *Ldlr*$^{-/-}$ mice with a deficiency in *apobec1*.[75] Taken together, these genetic studies suggest that thrombin contributes to atherosclerosis in mouse models.

Effect of Thrombin Inhibition on Atherosclerosis in Mice

The first study on the effect of the orally available thrombin inhibitor melagatran was performed in 2006.[76] Melagatran decreased progression of advanced lesions in *apoE*$^{-/-}$ mice on chow.[76] In addition, melagatran decreased inflammation and macrophages in the lesion and increased collagen and fibrous cap thickness, which is suggestive of a stable plaque phenotype. Similarly, dabigatran etexilate reduced atherosclerosis in *apoE*$^{-/-}$ mice on chow.[49] Moreover, 3 studies demonstrated that dabigatran etexilate (5.6–10 mg/g chow) reduced atherosclerosis in *apoE*$^{-/-}$ mice fed a Western diet.[46–48] These studies also showed that thrombin inhibition reduced inflammation, macrophage accumulation, and necrotic core volume while increasing fibrous cap thickness and improving endothelial function. In the cuff model, dabigatran etexilate reduced atherosclerosis in Western diet-fed *apoE*$^{-/-}$ mice expressing a mutant version of thrombomodulin with reduced anticoagulant activity.[50] In contrast, the thrombin inhibitor bivalirudin did not attenuate atherosclerosis.[77] However, bivalirudin has a short half-life and was administered daily via subcutaneous injection. Taken together, the majority of studies demonstrated that inhibition of thrombin decreased atherosclerosis and inflammatory mediators, such as IL-6 (interleukin-6), MCP-1 (monocyte chemoattractant protein 1), IFN-γ (interferon gamma), and TNF-α (tumor necrosis factor-α), in *apoE*$^{-/-}$ mice.

Effect of FXa Inhibition on Atherosclerosis in Mice

Low-dose rivaroxaban attenuated the development of atherosclerosis and inflammation in the aorta of 8-week-old male *apoE*$^{-/-}$ mice fed a Western diet for 20 weeks.[78] Interestingly, low-dose rivaroxaban (0.006 and 0.031 mg/g chow) did not induce plaque regression in female *apoE*$^{-/-}$ mice fed regular

chow for 26 weeks and then given rivaroxaban for an additional 26 weeks.[42,78] However, both doses of rivaroxaban reduced the expression of inflammatory mediators, such as TNF-α and IL-6.[42] Additionally, the 0.031 mg/g dose of rivaroxaban stabilized the plaque phenotype as reflected by a thicker fibrous cap, smaller necrotic cores, and more collagen.[42] We found that high-dose rivaroxaban (1.2 mg/g diet) reduced the development of atherosclerosis in 8-week-old female *Ldlr*$^{-/-}$ mice fed a Western diet for 14 weeks in the cuff model (J. Posma and H. Spronk, unpublished data, 2018). Additionally, we examined the effect a high-dose rivaroxaban (1.2 mg/g chow) on preexisting plaques by feeding female *apoE*$^{-/-}$ mice Western diet for 14 weeks and then administering rivaroxaban for 6 weeks in the cuff model. Rivaroxaban promoted regression of preexisting plaques (J. Posma and H. Spronk, unpublished data, 2018). These differences are likely because of the use of a different dose of rivaroxaban (1.2 versus 0.03 mg/g chow) with the higher dose more typical for studies with rivaroxaban.

Effect of Par1 Deficiency or Par1 Inhibition on Atherosclerosis in Mice

PAR1 expression is increased in human and mouse atherosclerotic lesions suggesting that it may play a role in atherosclerosis.[79] There are several studies that have investigated the role of PAR1 in atherosclerosis in the *apoE*$^{-/-}$ and *Ldlr*$^{-/-}$ models (Table 3). However, the data are inconsistent between the 2 models. In the *apoE*$^{-/-}$ mouse model with Western diet *Par1* deficiency reduced atherosclerosis, whereas *Par1* deficiency had no effect in the *Ldlr*$^{-/-}$ model fed Western diet.[79–81] Inhibition of PAR1 with the cell penetrating PAR1 pepducin PZ-128 also reduced atherosclerosis, macrophage content of plaques, and inflammation in *apoE*$^{-/-}$ mice fed a Western diet.[77] However, one must be cautious with the interpretation of this data because PZ-128 also inhibits signaling from a PAR1/PAR2 heterodimer.[82] In vitro studies indicated that the thrombin-PAR1 pathway inhibits cholesterol efflux in macrophages and vascular smooth muscle cells, and contributes to leukocyte migration into lesions.[80] These results suggest that PAR1 plays a role in the *apoE*$^{-/-}$ model but not in the *Ldlr*$^{-/-}$ model. Future studies should directly compare the phenotypes of *apoE*$^{-/-}$ mice treated with dabigatran etexilate and *apoE*$^{-/-}$ lacking *Par1*.

Thrombin also activates PAR4 and is the main thrombin receptor on mouse platelets.[19] Platelets have been shown to contribute to atherosclerosis in mice.[88] An early study found that *Par4* deficiency did not affect atherosclerosis in *apoE*$^{-/-}$ mice fed a Western diet.[89] In contrast, we observed a significant reduction in atherosclerosis in male *Ldlr*$^{-/-}$ mice lacking *Par4* fed a Western diet for 12 weeks (A.P. Owens 3rd and N. Mackman, unpublished data, 2018). This suggests that the contribution of the thrombin/PAR4 pathway is relatively mild and can only be detected in the *Ldlr*$^{-/-}$ model.

Effect of PAR2 Deficiency on Atherosclerosis in Mice

PAR2 expression is increased in human atherosclerotic plaques suggesting that it may contribute to plaque progression.[79] Several studies have determined the effect of *Par2* deficiency on atherosclerosis in mice (Table 4). *Par 2* deficiency was associated with reduced atherosclerosis, reduced inflammation, and increased plaque stability in *apoE*$^{-/-}$ mice fed a Western diet.[90] This phenotype was confirmed and extended by a later study.[43] Bone marrow transplantation experiments indicated that *Par2* on hematopoietic cells but not nonhematopoietic cells drove atherosclerosis.[43] Furthermore, in vitro studies showed that activation of PAR2 on macrophages increased inflammation. Additionally, the PAR2 pepducin inhibitor PZ-235 showed no effects on lesion development in *apoE*$^{-/-}$ mice fed a Western diet.[77] We found that a deficiency of *Par2* also attenuated atherosclerosis in *Ldlr*$^{-/-}$ mice fed a Western diet.[79] Mice lacking *Par2* had decreased expression of

Table 3. Effects of *Par1* Deficiency in Mouse Models of Inflammatory Disease

Animal Model	Major Findings in *Par1*$^{-/-}$	Reference
apoE$^{-/-}$ WD	Atherogenesis↓	80
Ldlr$^{-/-}$ WD	Atherogenesis↔	79
I/R-O	Remodeling↓	83
SCD		45
NAFLD	Inflammation↓, steatosis↓	84
NAFLD	Inflammation↓, steatosis↓	51
NAFLD	Inflammation↓	85
CVB3	Infection↑, myocarditis↑	53
IAV	Infection↑	53
IAV	Inflammation↓ survival↑	86
PF	Fibrosis↓, inflammation↓	87

apoE$^{-/-}$ indicates apolipoprotein E deficient; CVB3, coxsackievirus B3; IAV, influenza A virus; I/R-O, ischemia-reperfusion open chest; NAFLD, non-alcoholic fatty liver disease; PF, pulmonary fibrosis; SCD, sickle cell disease; and WD, Western diet.

Table 4. Effects of *Par2* Deficiency in Mouse Models of Inflammatory Disease

Animal Model	Major Findings in *Par2*$^{-/-}$	References
apoE$^{-/-}$ WD	Inflammation↓, atherogenesis↓	90
apoE$^{-/-}$ WD	Inflammation↓, atherogenesis↓	43
Ldlr$^{-/-}$ WD	Inflammation↓, atherogenesis↓	79
I/R-O	Infarct size↓, inflammation↓	91
LAD	Ejection fraction↑, remodeling↓	40
SCD	Inflammation↓	45
NAFLD	Glucose tolerance↑, insulin sensitivity↑, steatosis↓, inflammation↓	92,93
Diabetic nephropathy	Fibrosis↓, inflammation↓	94
CVB3	Infection↓, myocarditis↓	95
IAV	Survival↑	96
IAV	Survival↓	97

apoE$^{-/-}$ indicates apolipoprotein E deficient; CVB3, coxsackievirus B3; I/R-O, ischemia-reperfusion open chest; IAV, influenza A virus; LAD, left anterior descending; Ldlr$^{-/-}$, low-density lipoprotein receptor deficient; NAFLD, non-alcoholic fatty liver disease; SCD, sickle cell disease; and WD, Western diet.

the chemokines CCL2 and CXCL1 in the circulation.[79] However, in contrast to the studies with the *apoE*$^{-/-}$ mice, bone marrow transplantation experiments indicated that *Par2* on nonhematopoietic cells but not hematopoietic cells drove atherosclerosis in this model. In vitro studies indicated that activation of PAR2 on vascular smooth muscle cells induced *Ccl2* and *Cxcl1* expression and enhanced monocyte migration.[79] It is surprising that there are cell type differences in the role of PAR2 in the *apoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice. Additional studies are needed to compare the phenotypes of wild-type mice treated with rivaroxaban and *Par2*$^{-/-}$ mice in the 2 models.

Myocardial Infarction

Myocardial infarction is a leading cause of morbidity and mortality. The most commonly used models of myocardial infarction in mice are either permanent ligation of the left anterior descending (LAD) artery or temporary ligation of the LAD followed by reperfusion (ischemia-reperfusion model [I/R]).[40,98] I/R can be either an open-chest model (I/R-O) similar to LAD, where the opening of the chest precedes I/R on the same day,[98] or in a closed-chest model where the ligature is placed around the LAD without tying and mice allowed to recover for 5 to 7 days before ligation (I/R-C).[99] In the I/R-O model, the surgery contributes to the inflammatory response, whereas in the I/R-C model, most of the surgical inflammation has resolved at days 5 to 7.[99] The I/R models provide information on the role of different pathways involved in reperfusion injury.

Effect of Inhibition of Coagulation on Myocardial Infarction in Mice

We found that inhibiting the TF/FVIIa complex in mice with active site-inhibited FVIIa reduced infarct size in an I/R-C model that consisted of 60 minutes of ischemia and 2 hours of reperfusion.[100] This was associated with reduced leukocyte infiltration and decreased gene expression of inflammatory mediators, such as IL-6, intercellular adhesion molecule-1, and IL-1β.[100] Similarly, the thrombin inhibitor hirudin also reduced infarct size in the I/R-O model that consisted of 30 minutes of ischemia and 2 hours of reperfusion.[83] We determined the effect of rivaroxaban on infarct size in an I/R-C model by administering 2 doses of rivaroxaban via intravenous injection (1.6 mg/kg) 15 minutes after ischemia and 5 minutes after reperfusion. Rivaroxaban significantly reduced infarct size after 24 hours (J. Posma and H. Spronk, unpublished data, 2018).

In the LAD permanent ligation model, administration of rivaroxaban (0.5 mg/g chow) immediately after cardiac injury did not alter infarct size but reduced remodeling of the heart and preserved ejection fraction after 3 days and beyond compared with the placebo group.[40] Interestingly, no protection was observed when rivaroxaban treatment was started 3 days post-ligation. This suggests that FXa inhibition affects the acute inflammatory phase in this model. However, rivaroxaban treatment did not alter inflammatory mediators 2 days after ligation.[40] In this study, an open-chest model was used that promotes inflammation, which might have masked the effects of FXa inhibition on inflammation.

These studies indicate that coagulation proteases contribute to inflammation and infarct size after cardiac I/R injury. The protective effects seen with inhibition of coagulation after cardiac injury may be because of a combination of reduced fibrin deposition, activation of PARs and other downstream effects, such as generation of fibrin degradation products and platelet activation.

Effect of Par1 Deficiency on Myocardial Infarction in Mice

We found that *Par1* deficiency did not affect infarct size but reduced remodeling 2 weeks post-infarction in an I/R-O model (Table 3).[83] The lack of effect of PAR1 on infarct size may be because it mediates both pathological and protective pathways via APC. Indeed, exogenous APC was shown to reduce infarct size after I/R injury, and this effect was abolished in *Par1*$^{-/-}$ mice.[101,102] As stated above, *Par1* deficiency does not affect platelet activation in mice. However, *Par4* deficiency reduced infarct size in an I/R-O model.[103] These studies suggest that some of the beneficial effects of thrombin inhibition might be because of a reduction in PAR4 signaling.

Effect of Par2 Deficiency on Myocardial Infarction in Mice

We found that *Par2* deficiency attenuated infarct size in an I/R-O model consisting of 30 minutes of ischemia and 2 hours of reperfusion (Table 4).[91] In addition, hearts of *Par2*$^{-/-}$ mice had reduced levels of inflammatory mediators, such as IL-1β and TNF-α, and decreased remodeling compared with hearts from wild-type mice.[91] In the LAD permanent ligation model, *Par2*$^{-/-}$ mice had reduced remodeling and preserved ejection fraction after 28 days.[104] This result is similar to the protective effects of rivaroxaban in the wild-type mice.[40] Indeed, rivaroxaban did not provide any protection to *Par2*$^{-/-}$ mice in the LAD permanent ligation model.[40] However, one must be cautious in interpreting these results because FXa and PAR2 may play roles in parallel pathways that contribute to cardiac remodeling after myocardial infarction. For instance, administration of rivaroxaban would reduce levels of the fibrin degradation fragment E1, which has been shown to exacerbate I/R injury[4] (Figure). Additionally, activation of PAR2 by other protease would be abolished in *Par2*$^{-/-}$ mice.

Diet-Induced Obesity

Obesity is a global healthcare crisis with an estimated 34% of adults in the United States classified as obese.[105] Obesity leads to chronic activation of the coagulation cascade and is a risk factor for the development of metabolic syndrome.[84,106] High levels of TF are expressed in adipose tissue, and there is abundant fibrin deposition in adipose tissue.[51,107,108] The role of the coagulation cascade and PARs in diet-induced obesity has been studied using different mouse models that are associated with body weight gain, inflammation, macrophage recruitment to the adipose tissue, and insulin resistance.

Two studies from the Samad group found that *Par2*$^{-/-}$ mice and mice expressing a mutant form of TF that lacks the cytoplasmic domain had reduced weight gain in a diet-induced obesity model.[92,93] It was proposed that hematopoietic cell

TF-PAR2 signaling increases adipose inflammation, hepatic inflammation, hepatic macrophage recruitment, and steatosis, whereas nonhematopoietic cell TF-FVIIa-PAR2 signaling drives obesity.

We found that mice expressing low levels of TF exhibited significantly less body weight gain when fed a high-fat diet (HFD; 45% kcal fat) for 16 weeks compared with controls (Y. Hisada and N. Mackman, unpublished data, 2018). Notably, adipocyte size was increased in epididymal and subcutaneous fat in wild-type controls but not in low TF mice (Y. Hisada and N. Mackman, unpublished data, 2018). Similarly, low levels of TF expression in hematopoietic cells were also associated with significantly less body weight gain in *Ldlr*$^{-/-}$ mice fed a Western diet compared with controls.[106] Administration of dabigatran etexilate to C57BL/6J mice reduced body weight gain when fed an HFD.[51,52] Dabigatran also suppressed the progression of sequelae in mice with establish obesity.[51] In contrast, a deficiency of *Par1* did not affect diet-induced body weight gain.[51,84] This initiated a search for the effector molecule(s) downstream of thrombin that drove obesity. One obvious candidate was fibrinogen. A previous study found that a sequence in the fibrinogen γ-chain (390–396) binds to macrophages via αMβ2 and that mutation of this sequence abolished binding.[109] Strikingly, mice expressing this mutant fibrinogen Fibγ$^{390–396A}$ were protected from diet-induced body weight gain in a similar way to thrombin-inhibited wild-type mice.[51] Similar to the results we observed with low TF mice, the size of adipocytes was not increased in Fibγ$^{390–396A}$ mice fed an HFD.[51] In addition, Fibγ$^{390–396A}$ mice had reduced numbers of macrophages in the adipose tissue.[51] These results suggest that thrombin drives diet-induced obesity via fibrin-dependent inflammation in the adipose.

Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease is the liver manifestation of metabolic syndrome and is estimated to affect ≈25% of the Western population.[110] Mice fed a Western diet develop hepatic inflammation and fatty livers that is mainly because of the accumulation of triglycerides. This is referred to as steatosis. We found that low TF fed a Western diet for 16 weeks did not develop steatosis (Y. Hisada and N. Mackman, unpublished data, 2018). Similarly, low levels of TF expression in hematopoietic cells reduced hepatic steatosis in *Ldlr*$^{-/-}$ mice fed a Western diet.[84] *Par1* deficiency also reduced hepatic inflammation and steatosis in C57BL/6J mice fed a Western diet for 3 months.[84] Finally, Fibγ$^{390–396A}$ mice had reduced hepatic inflammation and steatosis when fed a Western diet compared with controls.[51] These results indicate that TF-thrombin-PAR1 and TF-thrombin-fibrin pathways contribute to hepatic inflammation and steatosis in mice fed a Western diet.

Diabetic Nephropathy

Diabetic nephropathy is the most common cause of end-stage renal disease in the United States.[111] In a mouse model of diabetic nephropathy, FXa inhibition by edoxaban attenuated progression of the disease.[94] This was associated with decreased expression of proinflammatory genes, such as *TNF-α*, and profibrotic genes, such as *TGF-β*. Interestingly, *Par2* deficiency gave a similar phenotype, and edoxaban did not provide any additional protection to *Par2*$^{-/-}$ mice.[94] The authors speculated that targeting FXa or PAR2 may reduce diabetic nephropathy in humans.

Sickle Cell Disease

A substitution of glutamic acid in normal hemoglobin for valine causes sickle cell disease. The mutant hemoglobin polymerizes and aggregates of hemoglobin tetramers leads to the formation of sickle red blood cells. Patients with sickle cell disease exhibit vaso-occlusion within postcapillary venules and have systemic inflammation and activation of coagulation.[112] The 2 most common mouse models of sickle cell disease (Berkley [BERK] and Townes) have the mouse hemoglobin genes replaced with their human counterparts.[113,114] BERK and Townes mice have severe anemia, systemic inflammation, and activation of coagulation.[115]

The role of the clotting cascade in sickle cell mice has been analyzed using genetic and pharmacological approaches. Inhibition of TF reduced both coagulation and the inflammatory mediator IL-6 in both BERK and Townes mice.[115] Moreover, reducing TF expression in nonhematopoietic cells to ≈1% in BERK mice reduced plasma IL-6, cardiac hypertrophy, and infiltration of neutrophils into the lungs but not activation of coagulation.[116] Deletion of TF in endothelial cells also reduced plasma IL-6 but not activation of coagulation.[45] Similarly, reducing circulating prothrombin to ≈10% of wild-type levels using an antisense oligonucleotide decreased early mortality in BERK mice.[117] In addition, a genetic reduction of prothrombin to ≈10% of wild-type levels in BERK mice reduced inflammation, endothelial dysfunction, and end-organ damage in the kidney, liver, and lung.[117]

Interestingly, short-term FXa inhibition with rivaroxaban, but not short-term thrombin inhibition with dabigatran, significantly reduced IL-6 plasma levels in sickle cell mice.[45] Similarly, *Par2*$^{-/-}$ mice but not *Par1*$^{-/-}$ mice with sickle cell bone marrow had reduced levels of plasma IL-6.[45] These results indicate that TF, FXa, and thrombin contribute to the activation of coagulation in sickle cell mice. In contrast, inflammation and end-organ dysfunction are more complex and seem to be driven by multiple pathways that include endothelial cell TF expression that activates PAR2 and also thrombin-dependent pathways. The different phenotypes observed in sickle cell mice may be because of the use of genetic versus pharmacological approaches and short- versus long-term studies.

Viral Infections

The blood coagulation cascade is activated in response to viral infection and can lead to disseminated intravascular coagulation.[118] Inhibition of the TF/FVIIa complex reduced inflammation and mortality in a primate model of Ebola hemorrhagic fever.[119] The roles of PAR1 and PAR2 in mouse models of viral infections are controversial.[118]

Coxsackievirus B3

We found that inhibition of either TF with an antibody or thrombin with dabigatran etexilate increased viral load and

myocarditis after infection of mice with coxsackievirus B3 (CVB3).[53] Similarly, *Par1*$^{-/-}$ mice exhibited increased CVB3-induced myocarditis (Table 3). TLR3 (Toll-like receptor 3) is one of the main receptors in the innate immune system that detects single-stranded RNA viruses, such as CVB3. TLR3 can be activated by the double-stranded RNA mimetic poly I:C. Importantly, we found that activation of PAR1 enhanced poly I:C induction of IFN-β expression in murine cardiac fibroblasts, suggesting that PAR1 contributes to the innate immune response to single-stranded RNA viral infection.[53] In complete contrast to the results with *Par1*$^{-/-}$ mice, *Par2*$^{-/-}$ mice were protected against CVB3-induced myocarditis.[95] We also found that administration of rivaroxaban (0.5 mg/g chow) to wild-type mice decreased CVB3-induced myocarditis (S. Antoniak and N. Mackman, unpublished data, 2018). Interestingly, murine cardiac fibroblasts lacking PAR2 had higher levels of IFN-β expression after stimulation with poly I:C compared with wild-type cells, suggesting that PAR2 negatively regulates TLR3-dependent expression of IFN-β.[95] Similarly, PAR2 inhibited TLR3-dependent expression of IFN-β in human epithelial cells.[96] These studies suggest that TLR3-dependent activation of antiviral pathways is positively and negatively regulated by PAR1 and PAR2, respectively.

Influenza A Virus

Influenza A virus (IAV) is a single-stranded RNA virus. Khoufache et al[86] found that *Par1* deficiency decreased inflammation and increased survival of mice infected with a mouse adapted H1N1 strain of IAV (Table 3). In contrast, we found that *Par1*$^{-/-}$ mice exhibited a decrease in the innate immune response and increase in virus genomes after IAV infection.[53] We have also observed increased mortality in *Par1*$^{-/-}$ mice compared with wild-type controls after IAV infection (S. Antoniak and N. Mackman, unpublished data, 2018). These variable results may be because of the use of a different dose of virus. Similarly, the results with *Par2*$^{-/-}$ are not consistent. Khoufache et al[97] observed that *Par2*$^{-/-}$ mice had increased inflammation and decreased survival after IAV infection. In contrast, the Vogel group found that *Par2*$^{-/-}$ mice exhibited increased survival compared with controls after IAV infection.[96] Similarly, administration of rivaroxaban (0.5 mg/g chow) to wild-type mice increased survival after IAV infection compared with controls (S. Antoniak and N. Mackman, unpublished data, 2018). Further studies are needed to determine the roles of coagulation proteases and PARs in IAV infection.

Other Viruses

PAR1 inhibition protected mice against respiratory syncytial virus and human metapneumovirus infection.[120] Similarly, thrombin inhibition with argatroban reduced the pathogenicity of the infection with no additional effect to PAR1 inhibition. In vitro studies with human A549 cells showed that PAR1 inhibition reduced the replication of respiratory syncytial virus and human metapneumovirus infection.[120] Further studies are needed to determine the roles of coagulation proteases and PARs in different viral infections.

Sepsis/Endotoxemia

Sepsis is induced by a systemic infection and activates the coagulation system. Endotoxemia is related to sepsis and is caused by endotoxins, most commonly bacterial lipopolysaccharide, in the blood. Lipopolysaccharide induces TF expression in monocytes.[121] Administration of lipopolysaccharide to mice leads to a rapid activation of coagulation. We found that mice expressing low levels of TF had less activation of coagulation and prolonged survival compared with controls in an endotoxemia model.[122] In a subsequent publication, we demonstrated endotoxemia-induced activation of coagulation was initiated by both hematopoietic and nonhematopoietic sources of TF.[123] Furthermore, inhibition of thrombin with hirudin prolonged survival but did not reduce inflammation. We also found that *Par1*- and *Par2*-deficient mice were not protected against endotoxemia.[122] An independent study also showed that a deficiency of *Par1*, *Par2*, or *Par4* did not affect inflammation or survival in an endotoxemia model that used different doses of lipopolysaccharide and both sexes.[124] In contrast to these 2 studies, 1 study reported protection of *Par1*-deficient mice in an endotoxemia model.[125] More recently, it was shown that mice expressing PAR1^{R41Q} that cannot be activated by thrombin were not protected in an *E coli*-induced pneumonia model.[28] These studies indicate a role for TF and thrombin in the activation of coagulation in endotoxemia, but the role of PARs in the inflammatory response in this model is controversial.

Idiopathic Pulmonary Fibrosis

The coagulation cascade plays a critical role in hemostasis in the lung but also contributes to fibroproliferative lung diseases, such as idiopathic pulmonary fibrosis. The ACE-IPF trial (Antioagulant Effectiveness in Idiopathic Fibrosis) investigated the effect of the anticoagulant warfarin in idiopathic pulmonary fibrosis but was stopped early because of excess risk of mortality.[126] Bleomycin induces lung injury and fibrosis in mice and is an established model of human pulmonary fibrosis. We found that bleomycin increased TF expression in the lungs of mice.[127] One study showed that administration of an FXa inhibitor ZK80734 to mice reduced bleomycin-induced lung injury in mice.[128] However, it is notable that 3 of 6 mice that received saline and ZK80734 and 3 of 9 mice that received bleomycin and ZK80734 were euthanized because of intraperitoneal hemorrhage after administration of the drug. PAR1 is highly expressed in cells associated with fibrotic foci in idiopathic pulmonary fibrosis suggesting that it may contribute to fibrosis.[129] Indeed, *Par1*$^{-/-}$ mice were protected from bleomycin-induced lung inflammation.[87] In vitro studies showed that FXa activated PAR1 on human adult lung fibroblasts.[128] PAR1 signaling leads to activation of TGF-β1, which is a key fibrotic mediator in many fibrotic conditions.

Conclusions

Activation of the blood coagulation leads to the generation of multiple coagulation proteases, fibrin deposition, proinflammatory fibrin degradation products, platelet activation, and PAR signaling (Figure). In atherosclerosis, inhibition of either FXa or thrombin reduces inflammation and lesion

development. Similarly, a deficiency of *Par2* reduces inflammation and atherosclerosis, but PAR2 seems to play different roles in the *apoE*$^{-/-}$ and *Ldlr*$^{-/-}$ models. *Par1* deficiency reduces atherosclerosis in the *apoE*$^{-/-}$ model, and this may be because of reduced cholesterol influx and monocyte migration into lesions. However, *Par1* deficiency had no effect in atherosclerosis in the *Ldlr*$^{-/-}$ model. In cardiac I/R injury, inhibition of either TF/FVIIa, FXa, or thrombin reduces inflammation and infarct size. Similarly, *Par2*$^{-/-}$ mice but not *Par1*$^{-/-}$ mice had reduced infarcts compared with controls. In diet-induced obesity mouse models, the TF/FVIIa-thrombin-fibrin pathway and PAR2-dependent pathways drive inflammation in adipose tissue. In a mouse model of sickle cell disease, the TF/FVIIa-FXa-PAR2 and TF-thrombin-PAR1 pathways drive inflammation and end-organ damage. The roles of PARs in viral infections are controversial. TLR3-dependent antiviral responses seem to be positively and negatively regulated by PAR1 and PAR2, respectively.

Further studies are needed with mice that express either mutant forms of the PARs or with cell type-specific deletion of PARs to elucidate how coagulation protease-PAR pathways contribute to different diseases.

Acknowledgments

We thank Drs Heitmeier, Luyendyk, Pawlinski, and Van Ryn for helpful comments.

Sources of Funding

This work was supported by funds from the John C. Parker Endowed Professorship, a grant from the National Institutes of Health (HL119523), and a grant from the Dutch Heart Foundation (Young Talent Program, CVON 2014-09).

Disclosures

N. Mackman is a consultant for Bayer. H. M. Spronk is a consultant for Bayer and has a research grant from Bayer.

References

1. Spronk HM, Govers-Riemslag JW, ten Cate H. The blood coagulation system as a molecular machine. *Bioessays*. 2003;25:1220–1228. doi: 10.1002/bies.10360
2. Mackman N, Tilley RE, Key NS. Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol*. 2007;27:1687–1693. doi: 10.1161/ATVBAHA.107.141911
3. Monroe DM, Hoffman M. What does it take to make the perfect clot? *Arterioscler Thromb Vasc Biol*. 2006;26:41–48. doi: 10.1161/01.ATV.0000193624.28251.83
4. Petzelbauer P, Zacharowski PA, Miyazaki Y, Friedl P, Wickenhauser G, Castellino FJ, Gröger M, Wolff K, Zacharowski K. The fibrin-derived peptide Bbeta15-42 protects the myocardium against ischemia-reperfusion injury. *Nat Med*. 2005;11:298–304. doi: 10.1038/nm1198
5. Flick MJ, Du X, Witte DP, Jirousková M, Soloviev DA, Busuttil SJ, Plow EF, Degen JL. Leukocyte engagement of fibrin(ogen) via the integrin receptor alphaMbeta2/Mac-1 is critical for host inflammatory response in vivo. *J Clin Invest*. 2004;113:1596–1606. doi: 10.1172/JCI20741
6. Koupenova M, Clancy L, Corkrey HA, Freedman JE. Circulating platelets as mediators of immunity, inflammation, and thrombosis. *Circ Res*. 2018;122:337–351. doi: 10.1161/CIRCRESAHA.117.310795
7. Camerer E, Huang W, Coughlin SR. Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proc Natl Acad Sci USA*. 2000;97:5255–5260.
8. Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J Thromb Haemost*. 2005;3:1800–1814. doi: 10.1111/j.1538-7836.2005.01377.x
9. Riewald M, Ruf W. Mechanistic coupling of protease signaling and initiation of coagulation by tissue factor. *Proc Natl Acad Sci USA*. 2001;98:7742–7747. doi: 10.1073/pnas.141126698
10. Rao LV, Pendurthi UR. Tissue factor-factor VIIa signaling. *Arterioscler Thromb Vasc Biol*. 2005;25:47–56. doi: 10.1161/01.ATV.0000151624.45775.13
11. Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A. PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell*. 2005;120:303–313. doi: 10.1016/j.cell.2004.12.018
12. Austin KM, Covic L, Kuliopulos A. Matrix metalloproteases and PAR1 activation. *Blood*. 2013;121:431–439. doi: 10.1182/blood-2012-09-355958
13. Sebastiano M, Momi S, Falcinelli E, Bury L, Hoylaerts MF, Gresele P. A novel mechanism regulating human platelet activation by MMP-2-mediated PAR1 biased signaling. *Blood*. 2017;129:883–895. doi: 10.1182/blood-2016-06-724245
14. Corvera CU, Déry O, McConalogue K, Böhm SK, Khitin LM, Caughey GH, Payan DG, Bunnett NW. Mast cell tryptase regulates rat colonic myocytes through proteinase-activated receptor 2. *J Clin Invest*. 1997;100:1383–1393. doi: 10.1172/JCI119658
15. Knecht W, Cottrell GS, Amadesi S, Mohlin J, Skåregärde A, Gedda K, Peterson A, Chapman K, Hollenberg MD, Vergnolle N, Bunnett NW. Trypsin IV or mesotrypsin and p23 cleave protease-activated receptors 1 and 2 to induce inflammation and hyperalgesia. *J Biol Chem*. 2007;282:26089–26100. doi: 10.1074/jbc.M703840200
16. Le Gall SM, Szabo R, Lee M, Kirchhofer D, Craik CS, Bugge TH, Camerer E. Matriptase activation connects tissue factor-dependent coagulation initiation to epithelial proteolysis and signaling. *Blood*. 2016;127:3260–3269. doi: 10.1182/blood-2015-11-683110
17. O'Brien PJ, Prevost N, Molino M, Hollinger MK, Woolkalis MJ, Woulfe DS, Brass LF. Thrombin responses in human endothelial cells. Contributions from receptors other than PAR1 include the transactivation of PAR2 by thrombin-cleaved PAR1. *J Biol Chem*. 2000;275:13502–13509.
18. McEachron TA, Pawlinski R, Richards KL, Church FC, Mackman N. Protease-activated receptors mediate crosstalk between coagulation and fibrinolysis. *Blood*. 2010;116:5037–5044. doi: 10.1182/blood-2010-06-293126
19. Nakanishi-Matsui M, Zheng YW, Sulciner DJ, Weiss EJ, Ludeman MJ, Coughlin SR. PAR3 is a cofactor for PAR4 activation by thrombin. *Nature*. 2000;404:609–613. doi: 10.1038/35007085
20. Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell*. 1991;64:1057–1068.
21. Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin SR. Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J Clin Invest*. 1999;103:879–887. doi: 10.1172/JCI6042
22. Kearon C, Akl EA, Comerota AJ, Prandoni P, Bounameaux H, Goldhaber SZ, Nelson ME, Wells PS, Gould MK, Dentali F, Crowther M, Kahn SR. Antithrombotic therapy for VTE disease: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2012;141:e419S–e496S.
23. Yeh CH, Hogg K, Weitz JI. Overview of the new oral anticoagulants: opportunities and challenges. *Arterioscler Thromb Vasc Biol*. 2015;35:1056–1065. doi: 10.1161/ATVBAHA.115.303397
24. Weitz JI, Harenberg J. New developments in anticoagulants: past, present and future. *Thromb Haemost*. 2017;117:1283–1288. doi: 10.1160/TH16-10-0807
25. Wagner NM, Dressel T, Schäfer K, Konstantinides S. Effect of the factor Xa inhibitor rivaroxaban on arterial thrombosis in wild-type and apolipoprotein E-deficient mice. *Thromb Res*. 2012;130:793–798. doi: 10.1016/j.thromres.2012.01.002
26. Iba T, Aihara K, Yamada A, Nagayama M, Tabe Y, Ohsaka A. Rivaroxaban attenuates leukocyte adhesion in the microvasculature and thrombus formation in an experimental mouse model of type 2 diabetes mellitus. *Thromb Res*. 2014;133:276–280. doi: 10.1016/j.thromres.2013.11.013
27. Shaya SA, Saldanha LJ, Vaezzadeh N, Zhou J, Ni R, Gross PL. Comparison of the effect of dabigatran and dalteparin on thrombus stability in a murine model of venous thromboembolism. *J Thromb Haemost*. 2016;14:143–152. doi: 10.1111/jth.13182
28. Sinha RK, Wang Y, Zhao Z, Xu X, Burnier L, Gupta N, Fernández JA, Martin G, Kupriyanov S, Mosnier LO, Zlokovic BV, Griffin JH. PAR1 biased signaling is required for activated protein C in vivo benefits in sepsis and stroke. *Blood*. 2018;131:1163–1171. doi: 10.1182/blood-2017-10-810895
29. Ebert J, Wilgenbus P, Teiber JF, Jurk K, Schwierczek K, Döhrmann M, Xia N, Li H, Spiecker L, Ruf W, Horke S. Paraoxonase-2 regulates coagulation

activation through endothelial tissue factor. *Blood*. 2018;131:2161–2172. doi: 10.1182/blood-2017-09-807040
30. van den Berg YW, Osanto S, Reitsma PH, Versteeg HH. The relationship between tissue factor and cancer progression: insights from bench and bedside. *Blood*. 2012;119:924–932. doi: 10.1182/blood-2011-06-317685
31. Rak J, Milsom C, Magnus N, Yu J. Tissue factor in tumour progression. *Best Pract Res Clin Haematol*. 2009;22:71–83. doi: 10.1016/j.beha.2008.12.008
32. Kasthuri RS, Taubman MB, Mackman N. Role of tissue factor in cancer. *J Clin Oncol*. 2009;27:4834–4838. doi: 10.1200/JCO.2009.22.6324
33. Zelaya H, Rothmeier AS, Ruf W. Tissue factor at the crossroad of coagulation and cell signaling. *J Thromb Haemost*. 2018;16:1941–1952.
34. Samama MM, Contant G, Spiro TE, Perzborn E, Le Flem L, Guinet C, Gourmelin Y, Rohde G, Martinoli JL. Laboratory assessment of rivaroxaban: a review. *Thromb J*. 2013;11:11. doi: 10.1186/1477-9560-11-11
35. Mueck W, Schwers S, Stampfuss J. Rivaroxaban and other novel oral anticoagulants: pharmacokinetics in healthy subjects, specific patient populations and relevance of coagulation monitoring. *Thromb J*. 2013;11:10. doi: 10.1186/1477-9560-11-10
36. Ketha H, Mills JR. To monitor dabigatran or not: a matter of patient safety. *Clin Chem*. 2015;61:691–693. doi: 10.1373/clinchem.2014.232207
37. Connolly SJ, Ezekowitz MD, Yusuf S, et al; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139–1151. doi: 10.1056/NEJMoa0905561
38. Reilly PA, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, Ezekowitz MD, Nehmiz G, Wang S, Wallentin L; RE-LY Investigators. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). *J Am Coll Cardiol*. 2014;63:321–328. doi: 10.1016/j.jacc.2013.07.104
39. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J*. 2008;22:659–661. doi: 10.1096/fj.07-9574LSF
40. Bode MF, Auriemma AC, Grover SP, Hisada Y, Rennie A, Bode WD, Vora R, Subramaniam S, Cooley B, Andrade-Gordon P, Antoniak S, Mackman N. The factor Xa inhibitor rivaroxaban reduces cardiac dysfunction in a mouse model of myocardial infarction. *Thromb Res*. 2018;167:128–134. doi: 10.1016/j.thromres.2018.05.015
41. Stangier J, Rathgen K, Stähle H, Gansser D, Roth W. The pharmacokinetics, pharmacodynamics and tolerability of dabigatran etexilate, a new oral direct thrombin inhibitor, in healthy male subjects. *Br J Clin Pharmacol*. 2007;64:292–303. doi: 10.1111/j.1365-2125.2007.02899.x
42. Zhou Q, Bea F, Preusch M, Wang H, Isermann B, Shahzad K, Katus HA, Blessing E. Evaluation of plaque stability of advanced atherosclerotic lesions in apo E-deficient mice after treatment with the oral factor Xa inhibitor rivaroxaban. *Mediators Inflamm*. 2011;432080. doi: 10.1155/2011/432080
43. Hara T, Phuong PT, Fukuda D, Yamaguchi K, Murata C, Nishimoto S, Yagi S, Kusunose K, Yamada H, Soeki T, Wakatsuki T, Imoto I, Shimabukuro M, Sata M. Protease-activated receptor-2 plays a critical role in vascular inflammation and atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. 2018;138:1706–1719. doi: 10.1161/CIRCULATIONAHA.118.033544
44. Goto M, Miura S, Suematsu Y, Idemoto Y, Takata K, Imaizumi S, Uehara Y, Saku K. Rivaroxaban, a factor Xa inhibitor, induces the secondary prevention of cardiovascular events after myocardial ischemia reperfusion injury in mice. *Int J Cardiol*. 2016;220:602–607. doi: 10.1016/j.ijcard.2016.06.212
45. Sparkenbaugh EM, Chantrathammachart P, Mickelson J, van Ryn J, Hebbel RP, Monroe DM, Mackman N, Key NS, Pawlinski R. Differential contribution of FXa and thrombin to vascular inflammation in a mouse model of sickle cell disease. *Blood*. 2014;123:1747–1756. doi: 10.1182/blood-2013-08-523936
46. Lee IO, Kratz MT, Schirmer SH, Baumhäkel M, Böhm M. The effects of direct thrombin inhibition with dabigatran on plaque formation and endothelial function in apolipoprotein E-deficient mice. *J Pharmacol Exp Ther*. 2012;343:253–257. doi: 10.1124/jpet.112.194837
47. Kadoglou NP, Moustardas P, Katsimpoulas M, Kapelouzou A, Kostomitsopoulos N, Schafer K, Kostakis A, Liapis CD. The beneficial effects of a direct thrombin inhibitor, dabigatran etexilate, on the development and stability of atherosclerotic lesions in apolipoprotein E-deficient mice: dabigatran etexilate and atherosclerosis. *Cardiovasc Drugs Ther*. 2012;26:367–374. doi: 10.1007/s10557-012-6411-3
48. Pingel S, Tiyerili V, Mueller J, Werner N, Nickenig G, Mueller C. Thrombin inhibition by dabigatran attenuates atherosclerosis in ApoE deficient mice. *Arch Med Sci*. 2014;10:154–160. doi: 10.5114/aoms.2014.40742
49. Preusch MR, Ieronimakis N, Wijelath ES, Cabbage S, Ricks J, Bea F, Reyes M, van Ryn J, Rosenfeld ME. Dabigatran etexilate retards the initiation and progression of atherosclerotic lesions and inhibits the expression of oncostatin M in apolipoprotein E-deficient mice. *Drug Des Devel Ther*. 2015;9:5203–5211. doi: 10.2147/DDDT.S86969
50. Borissoff JI, Otten JJT, Heeneman S, et al. Genetic and pharmacological modifications of thrombin formation in apolipoprotein e-deficient mice determine atherosclerosis severity and atherothrombosis onset in a neutrophil-dependent manner. *PLoS ONE*. 2013;8:e55784.
51. Kopec AK, Abrahams SR, Thornton S, Palumbo JS, Mullins ES, Divanovic S, Weiler H, Owens AP III, Mackman N, Goss A, van Ryn J, Luyendyk JP, Flick MJ. Thrombin promotes diet-induced obesity through fibrin-driven inflammation. *J Clin Invest*. 2017;127:3152–3166. doi: 10.1172/JCI92744
52. Kopec AK, Joshi N, Towery KL, Kassel KM, Sullivan BP, Flick MJ, Luyendyk JP. Thrombin inhibition with dabigatran protects against high-fat diet-induced fatty liver disease in mice. *J Pharmacol Exp Ther*. 2014;351:288–297. doi: 10.1124/jpet.114.218545
53. Antoniak S, Owens AP III, Baunacke M, et al. PAR-1 contributes to the innate immune response during viral infection. *J Clin Invest*. 2013;123:1310–1322. doi: 10.1172/JCI66125
54. van Ryn J, Stangier J, Haertter S, Liesenfeld KH, Wienen W, Feuring M, Clemens A. Dabigatran etexilate–a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thromb Haemost*. 2010;103:1116–1127. doi: 10.1160/TH09-11-0758
55. Harenberg J, Marx S, Wehling M. Head-to-head or indirect comparisons of the novel oral anticoagulants in atrial fibrillation: what's next? *Thromb Haemost*. 2012;108:407–409. doi: 10.1160/TH12-07-0463
56. Hapgood G, Butler J, Malan E, Chunilal S, Tran H. The effect of dabigatran on the activated partial thromboplastin time and thrombin time as determined by the Hemoclot thrombin inhibitor assay in patient plasma samples. *Thromb Haemost*. 2013;110:308–315. doi: 10.1160/TH13-04-0301
57. DeFeo K, Hayes C, Chernick M, Ryn JV, Gilmour SK. Use of dabigatran etexilate to reduce breast cancer progression. *Cancer Biol Ther*. 2010;10:1001–1008. doi: 10.4161/cbt.10.10.13236
58. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci USA*. 1989;86:2839–2843.
59. Ardissino D, Merlini PA, Ariëns R, Coppola R, Bramucci E, Mannucci PM. Tissue-factor antigen and activity in human coronary atherosclerotic plaques. *Lancet*. 1997;349:769–771. doi: 10.1016/S0140-6736(96)11189-2
60. Borissoff JI, Heeneman S, Kilinç E, Kassák P, Van Oerle R, Winckers K, Govers-Riemslag JW, Hamulyák K, Hackeng TM, Daemen MJ, ten Cate H, Spronk HM. Early atherosclerosis exhibits an enhanced procoagulant state. *Circulation*. 2010;122:821–830. doi: 10.1161/CIRCULATIONAHA.109.907121
61. Kato K, Elsayed YA, Namoto M, Nakagawa K, Sueishi K. Enhanced expression of tissue factor activity in the atherosclerotic aortas of cholesterol-fed rabbits. *Thromb Res*. 1996;82:335–347.
62. Hatakeyama K, Asada Y, Marutsuka K, Sato Y, Kamikubo Y, Sumiyoshi A. Localization and activity of tissue factor in human aortic atherosclerotic lesions. *Atherosclerosis*. 1997;133:213–219.
63. Daugherty A, Tall AR, Daemen MJAP, Falk E, Fisher EA, García-Cardeña G, Lusis AJ, Owens AP III, Rosenfeld ME, Virmani R; American Heart Association Council on Arteriosclerosis, Thrombosis and Vascular Biology; and Council on Basic Cardiovascular Sciences. Recommendation on design, execution, and reporting of animal atherosclerosis studies: a scientific statement from the American Heart Association. *Arterioscler Thromb Vasc Biol*. 2017;37:e131–e157. doi: 10.1161/ATV.0000000000000062
64. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb*. 1994;14:133–140.
65. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science*. 1992;258:468–471.
66. Davignon J. Apolipoprotein E and atherosclerosis: beyond lipid effect. *Arterioscler Thromb Vasc Biol*. 2005;25:267–269. doi: 10.1161/01.ATV.0000154570.50696.2c
67. Raffai RL, Loeb SM, Weisgraber KH. Apolipoprotein E promotes the regression of atherosclerosis independently of lowering plasma cholesterol levels. *Arterioscler Thromb Vasc Biol*. 2005;25:436–441. doi: 10.1161/01.ATV.0000152613.83243.12
68. Getz GS, Reardon CA. Do the Apoe-/- and Ldlr-/- mice yield the same insight on atherogenesis? *Arterioscler Thromb Vasc Biol*. 2016;36:1734–1741. doi: 10.1161/ATVBAHA.116.306874

69. Loeffen R, Spronk HM, ten Cate H. The impact of blood coagulability on atherosclerosis and cardiovascular disease. *J Thromb Haemost*. 2012;10:1207–1216. doi: 10.1111/j.1538-7836.2012.04782.x
70. Eitzman DT, Westrick RJ, Shen Y, Bodary PF, Gu S, Manning SL, Dobies SL, Ginsburg D. Homozygosity for factor V Leiden leads to enhanced thrombosis and atherosclerosis in mice. *Circulation*. 2005;111:1822–1825. doi: 10.1161/01.CIR.0000160854.75779.E8
71. Westrick RJ, Bodary PF, Xu Z, Shen YC, Broze GJ, Eitzman DT. Deficiency of tissue factor pathway inhibitor promotes atherosclerosis and thrombosis in mice. *Circulation*. 2001;103:3044–3046.
72. Tilley RE, Pedersen B, Pawlinski R, Sato Y, Erlich JH, Shen Y, Day S, Huang Y, Eitzman DT, Boisvert WA, Curtiss LK, Fay WP, Mackman N. Atherosclerosis in mice is not affected by a reduction in tissue factor expression. *Arterioscler Thromb Vasc Biol*. 2006;26:555–562. doi: 10.1161/01.ATV.0000202028.62414.3c
73. Xiao Q, Danton MJ, Witte DP, Kowala MC, Valentine MT, Degen JL. Fibrinogen deficiency is compatible with the development of atherosclerosis in mice. *J Clin Invest*. 1998;101:1184–1194. doi: 10.1172/JCI1461
74. Lou XJ, Boonmark NW, Horrigan FT, Degen JL, Lawn RM. Fibrinogen deficiency reduces vascular accumulation of apolipoprotein(a) and development of atherosclerosis in apolipoprotein(a) transgenic mice. *Proc Natl Acad Sci USA*. 1998;95:12591–12595.
75. Iwaki T, Sandoval-Cooper MJ, Brechmann M, Ploplis VA, Castellino FJ. A fibrinogen deficiency accelerates the initiation of LDL cholesterol-driven atherosclerosis via thrombin generation and platelet activation in genetically predisposed mice. *Blood*. 2006;107:3883–3891. doi: 10.1182/blood-2005-09-3780
76. Bea F, Kreuzer J, Preusch M, Schaab S, Isermann B, Rosenfeld ME, Katus H, Blessing E. Melagatran reduces advanced atherosclerotic lesion size and may promote plaque stability in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2006;26:2787–2792. doi: 10.1161/01.ATV.0000246797.05781.ad
77. Rana R, Huang T, Koukos G, Fletcher EK, Turner SE, Shearer A, Gurbel PA, Rade JJ, Kimmelstiel CD, Bliden KP, Covic L, Kuliopulos A. Noncanonical matrix metalloprotease 1-protease-activated receptor 1 signaling drives progression of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2018;38:1368–1380. doi: 10.1161/ATVBAHA.118.310967
78. Hara T, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Nishimoto S, Yagi S, Yamada H, Soeki T, Wakatsuki T, Shimabukuro M, Sata M. Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. *Atherosclerosis*. 2015;242:639–646. doi: 10.1016/j.atherosclerosis.2015.03.023
79. Jones SM, Mann A, Conrad K, Saum K, Hall DE, McKinney LM, Robbins N, Thompson J, Peairs AD, Camerer E, Rayner KJ, Tranter M, Mackman N, Owens AP III. PAR2 (Protease-Activated Receptor 2) deficiency attenuates atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2018;38:1271–1282. doi: 10.1161/ATVBAHA.117.310082
80. Raghavan S, Singh NK, Mani AM, Rao GN. Protease-activated receptor 1 inhibits cholesterol efflux and promotes atherogenesis via cullin 3-mediated degradation of the ABCA1 transporter. *J Biol Chem*. 2018;293:10574–10589. doi: 10.1074/jbc.RA118.003491
81. Ruf W. Proteases, protease-activated receptors, and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2018;38:1252–1254. doi: 10.1161/ATVBAHA.118.311139
82. Yoon H, Radulovic M, Wu J, Blaber SI, Blaber M, Fehlings MG, Scarisbrick IA. Kallikrein 6 signals through PAR1 and PAR2 to promote neuron injury and exacerbate glutamate neurotoxicity. *J Neurochem*. 2013;127:283–298. doi: 10.1111/jnc.12293
83. Pawlinski R, Pawlinski R, Tencati M, et al. Protease-activated receptor-1 contributes to cardiac remodeling and hypertrophy. *Circulation*. 2007;116:2298–2306.
84. Kassel KM, Owens AP III, Rockwell CE, Sullivan BP, Wang R, Tawfik O, Li G, Guo GL, Mackman N, Luyendyk JP. Protease-activated receptor 1 and hematopoietic cell tissue factor are required for hepatic steatosis in mice fed a Western diet. *Am J Pathol*. 2011;179:2278–2289. doi: 10.1016/j.ajpath.2011.07.015
85. Luyendyk JP, Sullivan BP, Guo GL, Wang R. Tissue factor-deficiency and protease activated receptor-1-deficiency reduce inflammation elicited by diet-induced steatohepatitis in mice. *Am J Pathol*. 2010;176:177–186. doi: 10.2353/ajpath.2010.090672
86. Khoufache K, Berri F, Nacken W, Vogel AB, Delenne M, Camerer E, Coughlin SR, Carmeliet P, Lina B, Rimmelzwaan GF, Planz O, Ludwig S, Riteau B. PAR1 contributes to influenza a virus pathogenicity in mice. *J Clin Invest*. 2013;123:206–214. doi: 10.1172/JCI61667
87. Howell DC, Johns RH, Lasky JA, Shan B, Scotton CJ, Laurent GJ, Chambers RC. Absence of proteinase-activated receptor-1 signaling affords protection from bleomycin-induced lung inflammation and fibrosis. *Am J Pathol*. 2005;166:1353–1365. doi: 10.1016/S0002-9440(10)62354-1
88. Linden MD, Jackson DE. Platelets: pleiotropic roles in atherogenesis and atherothrombosis. *Int J Biochem Cell Biol*. 2010;42:1762–1766. doi: 10.1016/j.biocel.2010.07.012
89. Hamilton JR, Cornelissen I, Mountford JK, Coughlin SR. Atherosclerosis proceeds independently of thrombin-induced platelet activation in ApoE-/- mice. *Atherosclerosis*. 2009;205:427–432. doi: 10.1016/j.atherosclerosis.2009.01.018
90. Zuo P, Zuo Z, Zheng Y, Wang X, Zhou Q, Chen L, Ma G. Protease-activated receptor-2 deficiency attenuates atherosclerotic lesion progression and instability in apolipoprotein E-deficient mice. *Front Pharmacol*. 2017;8:647. doi: 10.3389/fphar.2017.00647
91. Antoniak S, Rojas M, Spring D, Bullard TA, Verrier ED, Blaxall BC, Mackman N, Pawlinski R. Protease-activated receptor 2 deficiency reduces cardiac ischemia/reperfusion injury. *Arterioscler Thromb Vasc Biol*. 2010;30:2136–2142.
92. Badeanlou L, Furlan-Freguia C, Yang G, Ruf W, Samad F. Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation. *Nat Med*. 2011;17:1490–1497. doi: 10.1038/nm.2461
93. Wang J, Chakrabarty S, Bui Q, Ruf W, Samad F. Hematopoietic tissue factor-protease-activated receptor 2 signaling promotes hepatic inflammation and contributes to pathways of gluconeogenesis and steatosis in obese mice. *Am J Pathol*. 2015;185:524–535. doi: 10.1016/j.ajpath.2014.10.008
94. Oe Y, Hayashi S, Fushima T, Sato E, Kisu K, Sato H, Ito S, Takahashi N. Coagulation factor Xa and protease-activated receptor 2 as novel therapeutic targets for diabetic nephropathy. *Arterioscler Thromb Vasc Biol*. 2016;36:1525–1533. doi: 10.1161/ATVBAHA.116.307883
95. Weithauser A, Bobbert P, Antoniak S, Böhm A, Rauch BH, Klingel K, Savvatis K, Kroemer HK, Tschope C, Stroux A, Zeichhardt H, Poller W, Mackman N, Schultheiss HP, Rauch U. Protease-activated receptor-2 regulates the innate immune response to viral infection in a coxsackievirus B3-induced myocarditis. *J Am Coll Cardiol*. 2013;62:1737–1745. doi: 10.1016/j.jacc.2013.05.076
96. Nhu QM, Shirey K, Teijaro JR, Farber DL, Netzel-Arnett S, Antalis TM, Fasano A, Vogel SN. Novel signaling interactions between proteinase-activated receptor 2 and Toll-like receptors in vitro and in vivo. *Mucosal Immunol*. 2010;3:29–39. doi: 10.1038/mi.2009.120
97. Khoufache K, LeBouder F, Morello E, Laurent F, Riffault S, Andrade-Gordon P, Boullier S, Rousset P, Vergnolle N, Riteau B. Protective role for protease-activated receptor-2 against influenza virus pathogenesis via an IFN-gamma-dependent pathway. *J Immunol*. 2009;182:7795–7802. doi: 10.4049/jimmunol.0803743
98. Palazzo AJ, Jones SP, Girod WG, Anderson DC, Granger DN, Lefer DJ. Myocardial ischemia-reperfusion injury in CD18- and ICAM-1-deficient mice. *Am J Physiol*. 1998;275(6 pt 2):H2300–H2307.
99. Jong WM, Reitsma PH, ten Cate H, de Winter RJ. Modified two-step model for studying the inflammatory response during myocardial ischemia and reperfusion in mice. *Comp Med*. 2003;53:522–526.
100. Loubele ST, Spek CA, Leenders P, van Oerle R, Aberson HL, van der Voort D, Hamulyák K, Petersen LC, Spronk HM, ten Cate H. Active site inhibited factor VIIa attenuates myocardial ischemia/reperfusion injury in mice. *J Thromb Haemost*. 2009;7:290–298. doi: 10.1111/j.1538-7836.2008.03232.x
101. Loubele ST, Spek CA, Leenders P, van Oerle R, Aberson HL, Hamulyák K, Ferrell G, Esmon CT, Spronk HM, ten Cate H. Activated protein C protects against myocardial ischemia/ reperfusion injury via inhibition of apoptosis and inflammation. *Arterioscler Thromb Vasc Biol*. 2009;29:1087–1092. doi: 10.1161/ATVBAHA.109.188656
102. Nazir S, Gadi I, Al-Dabet MM, et al. Cytoprotective activated protein C averts Nlrp3 inflammasome-induced ischemia-reperfusion injury via mTORC1 inhibition. *Blood*. 2017;130:2664–2677. doi: 10.1182/blood-2017-05-782102
103. Kolpakov MA, Rafiq K, Guo X, Hooshdaran B, Wang T, Vlasenko L, Bashkirova YV, Zhang X, Chen X, Iftikhar S, Libonati JR, Kunapuli SP, Sabri A. Protease-activated receptor 4 deficiency offers cardioprotection after acute ischemia reperfusion injury. *J Mol Cell Cardiol*. 2016;90:21–29. doi: 10.1016/j.yjmcc.2015.11.030

104. Antoniak S, Sparkenbaugh EM, Tencati M, Rojas M, Mackman N, Pawlinski R. Protease activated receptor-2 contributes to heart failure. *PLoS ONE*. 2013;8:e81733.
105. Li C, Balluz LS, Okoro CA, Strine TW, Lin JM, Town M, Garvin W, Murphy W, Bartoli W, Valluru B; Centers for Disease Control and Prevention (CDC). Surveillance of certain health behaviors and conditions among states and selected local areas—behavioral risk factor surveillance system, United States, 2009. *MMWR Surveill Summ*. 2011;60:1–250.
106. Owens AP III, Passam FH, Antoniak S, et al. Monocyte tissue factor-dependent activation of coagulation in hypercholesterolemic mice and monkeys is inhibited by simvastatin. *J Clin Invest*. 2012;122:558–568. doi: 10.1172/JCI58969
107. Samad F, Pandey M, Loskutoff DJ. Tissue factor gene expression in the adipose tissues of obese mice. *Proc Natl Acad Sci USA*. 1998;95:7591–7596.
108. Samad F, Ruf W. Inflammation, obesity, and thrombosis. *Blood*. 2013;122:3415–3422. doi: 10.1182/blood-2013-05-427708
109. Flick MJ, Du X, Degen JL. Fibrin(ogen)-alpha M beta 2 interactions regulate leukocyte function and innate immunity in vivo. *Exp Biol Med (Maywood)*. 2004;229:1105–1110.
110. Sayiner M, Koenig A, Henry L, Younossi ZM. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clin Liver Dis*. 2016;20:205–214. doi: 10.1016/j.cld.2015.10.001
111. Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, Levey AS. Prevalence of chronic kidney disease in the United States. *JAMA*. 2007;298:2038–2047. doi: 10.1001/jama.298.17.2038
112. Ataga KI, Key NS. Hypercoagulability in sickle cell disease: new approaches to an old problem. *Hematology Am Soc Hematol Educ Program*. 2007;2007:91–96.
113. Pászty C, Brion CM, Manci E, Witkowska HE, Stevens ME, Mohandas N, Rubin EM. Transgenic knockout mice with exclusively human sickle hemoglobin and sickle cell disease. *Science*. 1997;278:876–878.
114. Wu LC, Sun CW, Ryan TM, Pawlik KM, Ren J, Townes TM. Correction of sickle cell disease by homologous recombination in embryonic stem cells. *Blood*. 2006;108:1183–1188. doi: 10.1182/blood-2006-02-004812
115. Chantrathammachart P, Mackman N, Sparkenbaugh E, Wang JG, Parise LV, Kirchhofer D, Key NS, Pawlinski R. Tissue factor promotes activation of coagulation and inflammation in a mouse model of sickle cell disease. *Blood*. 2012;120:636–646. doi: 10.1182/blood-2012-04-424143
116. Sparkenbaugh EM, Chantrathammachart P, Chandarajoti K, Mackman N, Key NS, Pawlinski R. Thrombin-independent contribution of tissue factor to inflammation and cardiac hypertrophy in a mouse model of sickle cell disease. *Blood*. 2016;127:1371–1373. doi: 10.1182/blood-2015-11-681114
117. Arumugam PI, Mullins ES, Shanmukhappa SK, Monia BP, Loberg A, Shaw MA, Rizvi T, Wansapura J, Degen JL, Malik P. Genetic diminution of circulating prothrombin ameliorates multiorgan pathologies in sickle cell disease mice. *Blood*. 2015;126:1844–1855. doi: 10.1182/blood-2015-01-625707
118. Antoniak S, Mackman N. Multiple roles of the coagulation protease cascade during virus infection. *Blood*. 2014;123:2605–2613. doi: 10.1182/blood-2013-09-526277
119. Geisbert TW, Hensley LE, Jahrling PB, Larsen T, Geisbert JB, Paragas J, Young HA, Fredeking TM, Rote WE, Vlasuk GP. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet*. 2003;362:1953–1958. doi: 10.1016/S0140-6736(03)15012-X
120. Lê VB, Riteau B, Alessi MC, Couture C, Jandrot-Perrus M, Rhéaume C, Hamelin MÈ, Boivin G. Protease-activated receptor 1 inhibition protects mice against thrombin-dependent respiratory syncytial virus and human metapneumovirus infections. *Br J Pharmacol*. 2018;175:388–403. doi: 10.1111/bph.14084
121. Gregory SA, Morrissey JH, Edgington TS. Regulation of tissue factor gene expression in the monocyte procoagulant response to endotoxin. *Mol Cell Biol*. 1989;9:2752–2755.
122. Pawlinski R, Pedersen B, Schabbauer G, Tencati M, Holscher T, Boisvert W, Andrade-Gordon P, Frank RD, Mackman N. Role of tissue factor and protease-activated receptors in a mouse model of endotoxemia. *Blood*. 2004;103:1342–1347. doi: 10.1182/blood-2003-09-3051
123. Pawlinski R, Wang JG, Owens AP III, Williams J, Antoniak S, Tencati M, Luther T, Rowley JW, Low EN, Weyrich AS, Mackman N. Hematopoietic and nonhematopoietic cell tissue factor activates the coagulation cascade in endotoxemic mice. *Blood*. 2010;116:806–814. doi: 10.1182/blood-2009-12-259267
124. Camerer E, Cornelissen I, Kataoka H, Duong DN, Zheng YW, Coughlin SR. Roles of protease-activated receptors in a mouse model of endotoxemia. *Blood*. 2006;107:3912–3921. doi: 10.1182/blood-2005-08-3130
125. Niessen F, Schaffner F, Furlan-Freguia C, Pawlinski R, Bhattacharjee G, Chun J, Derian CK, Andrade-Gordon P, Rosen H, Ruf W. Dendritic cell PAR1-S1P3 signalling couples coagulation and inflammation. *Nature*. 2008;452:654–658. doi: 10.1038/nature06663
126. Noth I, Anstrom KJ, Calvert SB, de Andrade J, Flaherty KR, Glazer C, Kaner RJ, Olman MA; Idiopathic Pulmonary Fibrosis Clinical Research Network (IPFnet). A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2012;186:88–95. doi: 10.1164/rccm.201202-0314OC
127. Olman MA, Mackman N, Gladson CL, Moser KM, Loskutoff DJ. Changes in procoagulant and fibrinolytic gene expression during bleomycin-induced lung injury in the mouse. *J Clin Invest*. 1995;96:1621–1630. doi: 10.1172/JCI118201
128. Scotton CJ, Krupiczojc MA, Königshoff M, Mercer PF, Lee YC, Kaminski N, Morser J, Post JM, Maher TM, Nicholson AG, Moffatt JD, Laurent GJ, Derian CK, Eickelberg O, Chambers RC. Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. *J Clin Invest*. 2009;119:2550–2563. doi: 10.1172/JCI33288
129. Mercer PF, Johns RH, Scotton CJ, Krupiczojc MA, Königshoff M, Howell DC, McAnulty RJ, Das A, Thorley AJ, Tetley TD, Eickelberg O, Chambers RC. Pulmonary epithelium is a prominent source of proteinase-activated receptor-1-inducible CCL2 in pulmonary fibrosis. *Am J Respir Crit Care Med*. 2009;179:414–425. doi: 10.1164/rccm.200712-1827OC