Protective and pathological roles of tissue factor in the heart

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Summary
Tissue factor (TF) is expressed in the heart where it is required for haemostasis. High levels of TF are also expressed in atherosclerotic plaques and likely contribute to atherothrombosis after plaque rupture. Indeed, risk factors for atherothrombosis, such as diabetes, hypercholesterolaemia, smoking and hypertension, are associated with increased TF expression in circulating monocytes, macrophages and plasma. Several therapies that reduce atherothrombosis, such as statins, ACE inhibitors, beta-blockers and anti-platelet drugs, are associated with reduced TF expression. In addition to its haemostatic and pro-thrombotic functions, the TF:FVIIa complex and downstream coagulation proteases activate cells by cleavage of protease-activated receptors (PARs). In mice, deficiencies in either PAR-1 or PAR-2 reduce cardiac remodelling and heart failure after ischaemia-reperfusion injury. This suggests that inhibition of coagulation proteases and PARs may be protective in heart attack patients. In contrast, the TF/thrombin/PAR-1 pathway is beneficial in a mouse model of Coxsackievirus B3-induced viral myocarditis. We found that stimulation of PAR-1 increases the innate immune response by enhancing TLR3-dependent IFN-β expression. Therefore, inhibition of the TF/thrombin/PAR-1 pathway in patients with viral myocarditis could have detrimental effects. Conclusion: The TF:FVIIa complex has both protective and pathological roles in the heart.

Schlüsselwörter
Tissue Factor, Hämostase, Atherothrombose, Kardiales Remodelling

Zusammenfassung

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TF and tissue factor pathway inhibitor

Tissue factor (TF) is the transmembrane cellular receptor for FVII/FVIIa. The TF:FVIIa complex activates FX and FIX which leads to thrombin generation, fibrin deposition and platelet activation (1). TF is essential for haemostasis but can trigger thrombosis (2).

Tissue factor pathway inhibitor (TFPI) is the natural inhibitor of TF.

Its primary source is endothelial cells but it is also expressed by several other cell types, such as platelets, monocytes, vascular smooth muscle cells and fibroblasts (6).
TFPI inhibits the TF: FVIIa complex in a FXa-dependent manner (6).

Healthy individuals do not express significant levels of TF on monocytes, microparticles (MPs) or in plasma. MPs are sub-micron plasma membrane vesicles that are released from activated and apoptotic cells (7, 8). However, TF is also expressed by activated monocytes and on monocyte-derived MPs as part of the innate immune response to injury and infection (9). MPs are easy to isolate from plasma and TF-positive MPs may represent a good biomarker of a pro-thrombotic state (10-12). After total knee arthroplasty, TF expression is increased in monocytes and in cell-free plasma on MPs (13). It should be noted, however, that MP TF represents only ~5% of the TF in blood after total knee arthroplasty.

There are a variety of commercial and non-commercial assays that are claimed to measure levels of TF in plasma. While some assays measure TF plasma antigen, others measure TF activity in plasma or MPs. Only two studies have simultaneously compared different TF assays (14, 15).

In our opinion, activity-based assays are a more reliable way to measure the very low levels of TF in plasma than antigen-based assays (16, 17).

We advise the reader that a limitation of this review is that some of the cited studies used antigen-based TF assays that may not accurately quantitate levels of TF in the blood. In this review we will discuss the protective role of TF in the heart in haemostasis and the innate immune response, as well as its pathological roles in atherothrombosis and cardiac remodelling.

Heart haemostasis

TF is constitutively expressed in the heart by cardiomyocytes and cardiac fibroblasts (18) where it appears to provide additional haemostatic protection beyond perivascular TF. We believe that blood vessels particularly in mechanically active organs, such as the heart or lungs, are constantly damaged, which will lead to detectable bleeding without normal haemostatic protection. For instance, in mice expressing very low levels of TF, heart haemostasis is severely compromised (19, 20). Haemorrhage into the myocardium appears to precede haemosiderin deposition and fibrosis in the hearts of these mice at three months of age and beyond (19). The haemostatic defect first appears in sub-epicardial and perivascular regions of the heart and then spreads throughout the whole heart (19). Importantly, heart haemostasis can be restored in low TF mice by overexpression of TF in cardiomyocytes (19, 21). We also generated mice that had the TF gene selectively deleted in cardiomyocytes (21). Under normal conditions, these mice exhibit only a mild haemostatic defect but this is dramatically worsened after treatment with the beta-adrenergic agonist isoproterenol, that increases heart rate and contractility (21). These data strongly suggest that cardiomyocyte and cardiac fibroblast TF are essential to maintain heart haemostasis (Fig. 1).

TF and atherothrombosis

Acute myocardial ischaemia is most frequently caused by the rupture of a vulnerable atherosclerotic plaque resulting in unstable angina (UA) or myocardial infarction (MI) (22-24). Infrequently, other mechanisms, such as vasospasm, dissection or supply-demand imbalance can lead to acute ischemia (24, 25). For diagnostic and treatment purposes MI can be further subdivided by electrocardiographic criteria into ST-elevation myocardial infarction (STEMI) and non-STEMI (24, 26, 27). Importantly, STEMI carries a higher short-term mortality compared to non-STEMI (24, 26, 27). During plaque rupture, platelets are activated (28) and coagulation is initiated when the blood comes in contact with TF present in the lipid core of the plaque (29-31), resulting in the formation of an intracoronary thrombus. One study showed that the thrombogenicity of human atherosclerotic plaques is directly related to their TF content (30). Thrombi of STEMI patients contain large amounts of TF predominantly within areas of platelet aggregates and monocytes (32). Moreover, levels of TF protein are increased in the plasma of patients with UA or MI compared to patients with stable angina (33-37). In STEMI, TF-positive MPs are increased in the stenosed artery compared to the peripheral blood (38). Monocyte-derived MPs were identified as a major source of plasma TF activity in acute myocardial ischaemia (39, 40). Increased plasma TF antigen has also been shown to be independently associated in UA patients with higher revascularization rates (41), and in MI patients with higher reinfarction rates and mortality (39, 42, 43). Levels of plasma TF antigen measured in STEMI patients just prior to primary percutaneous coronary intervention (PCI) is associated with long-term ischaemic events after a median follow up of one year (44). However, as noted above these studies rely on antigen-based assays to measure levels of TF in plasma.

As expected, TFPI inhibits TF-dependent thrombogenicity of human atherosclerotic and lipid-rich plaques in human arterial segments in vitro (45), and in a porcine model in vivo (46). In the acute phase STEMI patients show lower levels of TFPI compared to non-STEMI patients, which may lead to enhanced thrombosis at the culprit lesion (47).

A study with atherosclerotic ApoE-/-mice showed that a 50% reduction of TFPI was associated with increased thrombosis in a carotid artery model (48). More importantly, two recent studies that involved disruption of plaques in ApoE-/-mice showed that inhibition of TF reduced the size of the early thrombus (49). In contrast, inhibition of FXII or a reduction in FXI levels limited the thrombus at the amplification phase of the reaction (49). This suggests that both the extrinsic (TF/FVIIa) and intrinsic (FXII/FXI/FIX) pathways likely contribute to atherothrombosis in humans (50) (Fig. 1).

Risk factors for atherothrombosis are associated with increased TF

It is interesting to note that several of the risk factors for acute myocardial ischaemia increase TF expression, whereas the majority of the therapies that reduce mortality decrease TF expression (Fig. 1).
Diabetes mellitus

In vitro, high glucose concentrations increase TF expression in thrombin-stimulated human endothelial cells. Chronic hyperglycaemia leads to the formation of advanced glycation end-products (AGE), which have been shown to induce TF expression in endothelial cells by activation of the receptor for AGE (RAGE) (51) and the transcription factor NFκB (52). Patients with diabetes have increased levels of plasma TF antigen (53, 54) and improvement of glycaemic control reduces levels of plasma TF antigen (54). Diabetic ApoE/-/- mice exhibit increased vascular expression of RAGE and TF, and blockade of RAGE decreases TF expression in the aorta of these mice (55). We have shown that the transcription factor Egr-1 positively regulates TF gene expression (56–59). Interestingly, insulin has been shown to lower levels of AGE and TF in human subjects, which may be in part mediated by reducing levels of Egr-1 (60). Valsartan, an AT1 receptor blocker (ARB), also reduces Egr-1 and TF expression in streptozotocin-induced diabetic mice (61).

Hypercholesterolaemia

Oxidized LDL (oxLDL) induces TF expression in endothelial cells (62), monocytes (63), macrophages (64) and vascular smooth muscle cells (65) in vitro. We also found that a genetic deficiency of TF in haematopoietic cells reduced the activation of coagulation in hypercholesterolaemic mice (63). These data illustrate the important role of haematopoietic cell TF in inducing a pro-coagulant state in hypercholesterolaemia. Consistent with these findings, we showed that patients, monkeys and mice with elevated levels of oxLDL in plasma have increased MP TF activity (63).

Smoking

In vitro, cigarette smoke extract induces TF mRNA and protein expression and the generation of procoagulant MPs by human monocytes (66). Cigarette smoke also increases TF expression in atherosclerotic plaques of ApoE/-/- mice (67). In humans, plasma TF antigen is increased after exposure to cigarette smoke and a strong correlation is observed between the number of cigarettes smoked and the levels of TF antigen in the plasma (54).

Hypertension

Angiotensin II, which is elevated in hypertensive subjects, induces TF expression in monocytes (68), endothelial cells (69) and vascular smooth muscle cells (70, 71) through the AT1 receptor (68, 70). In addition, TF plasma antigen is increased in patients with hypertension compared to normotensive controls and can be lowered by antihypertensive treatment (72). Inhibition of angiotensin II generation by angiotensin-converting enzyme (ACE) inhibitors (73) and inhibition of the AT1 receptor (68, 71, 74) decrease TF plasma activity in hypertensive patients.

Atheroprotective treatments

Statins prevent recurrent thrombotic events after acute myocardial ischaemia if given early after the ischaemic event (75, 76) and have been shown to reduce mortality (77, 78). Statins have anti-inflammatory and anti-thrombotic properties (79, 80). As discussed above, patients with acute myocardial ischaemia have elevated levels of monocyte-derived MPs and TF-positive MPs (38, 40), suggesting a pro-coagulant role for TF in this scenario. In vitro, simvastatin inhibits TF expression in human endothelial cells and monocytes (63, 81). In ApoE-/- mice, simvastatin and rosuvastatin inhibit TF expression independent of plasma lipid levels, possibly by inhibition of Egr-1 and NFκB (79, 82). Cerivastatin reduces TF expression in atherosclerotic lesions of hypercholesterolaemic rabbits (83). Recently, we found that simvastatin reduces monocyte TF expression, MP TF activity and coagulation in hypercholesterolemic mice and monkeys without affecting plasma lipid levels (63). In humans with elevated hs-CRP but normal LDL levels, rosuvastatin significantly reduces the incidence of cardiovascular events and symptomatic venous thromboembolism and it was speculated that this may be due, in part, to inhibition of monocyte TF expression (84).
ACE inhibitors and ARBs

ACE inhibitors and ARBs inhibit the renin-angiotensin-aldosterone system. ACE inhibitors have been shown to prevent recurrent MI (85–88). Most of the protective effects of these medications are generally thought to be achieved by lowering blood pressure and reducing pathological cardiac remodelling. In addition, they appear to possess anti-thrombotic and anti-inflammatory properties (89). Monocytes were identified to be the major source of TF within the coronary plaque (90, 91) and they were found to be capable of producing ACE (92, 93). Placebo controlled trials showed a decrease in TF antigen levels in the plasma in MI patients treated with the ACE inhibitor enalapril (73, 94). Several ARBs have been shown to lower plasma TF activity in hypertensive subjects with the strongest effect being observed with candesartan (74). These findings were further corroborated by another study that showed the ACE inhibitor captopril and the ARB losartan decrease TF expression in endotoxin-stimulated human monocytes (95). These results are consistent with the hypothesis that TF expression is regulated by endogenous angiotensin II and that ACE inhibitors and ARBs have blood-pressure-independent effects on TF expression.

Beta-blockers

Beta-blockers reduce mortality after MI (96–98). Carvedilol, a nonselective beta-adrenoceptor antagonist with alpha 1-adrenoceptor blockade action, suppresses LPS induced TF expression in human monocytes (99). This appears to be due to a reduction of Egr-1 independent of carvedilol’s adrenoreceptor inhibitory action. Furthermore, treatment of rats with MI after MI with carvedilol and the ARB irbesartan lowered TF mRNA and protein expression in the myocardium and decreased infarct size to a greater extent than treatment with either drug alone (100). These findings suggest that the beneficial effects of beta-blockers may be due, in part, to lowering TF. This would be expected to decrease the thrombus size in the acute setting and the re-infarction rate in the subacute setting. Moreover, these effects are likely achieved through pathways that are distinct from those utilized by ARBs given that the effect was additive.

Antiplatelet agents

Platelets do not appear to express significant levels of functional TF (101, 102). However, platelet activation enhances TF expression by LPS-stimulated monocytes (103, 104). Therefore it is conceivable that antiplatelet agents could be beneficial in lowering plasma TF activity. We and others have shown that acetylsalicylic acid (ASA) inhibits endotoxin-induced TF mRNA and protein expression in monocytes in vitro (105,106). ASA has also been shown to decrease plasma TF antigen in mice after exposure to cigarette smoke (67). The ADP receptor antagonist clopidogrel reduces platelet-induced TF expression in endothelial cells while ASA is ineffective in this setting (107). Similarly, plasma TF activity was reduced in patients with peripheral artery disease after a two-week treatment with clopidogrel but not ASA (108). Finally, the GPIIb/IIIa antagonist abciximab suppresses monocyte TF expression by reducing platelet monocyte cross-talk as shown in vitro (109) and in patients undergoing carotid angioplasty with stenting (110). These results suggest that antiplatelet agents lower plasma TF activity and do this primarily by inhibiting platelet-induced monocyte activation.

PCI

PCI is the preferred treatment for patients with STEMI and reduces mortality (111, 112). Reports regarding TF levels after PCI are somewhat contradictory. One group found increased plasma TF antigen levels after PCI that returned to baseline after 24 hours in patients presenting with MI (113). However, another group did not find a change in whole-blood TF activity after PCI in patients with stable angina (114). These differences may be explained by different patient populations and/or the use of different assays. Unfortunately, no long term data for TF levels in patients after undergoing PCI is available. One would expect an increase in plasma TF immediately after PCI due to its release from the vessel wall and plaque.

TF/FVIIa inhibitors

Initially it was thought that TF represented a good target for anticoagulant therapy because it is at the top of the clotting cascade and is expressed at high levels around blood vessels so bleeding would be unlikely. However, TF plays an essential role in haemostasis and genetic deficiency or pharmacological inhibition is associated with bleeding (20, 115).

Three different anti-human TF antibodies have been developed that inhibit the TF:FVIIa complex. The murine monoclonal antibody TF8–5G9 binds the preformed TF:FVIIa complex and blocks FX/FXII binding and activation (116, 117). In endotoxin-treated chimpanzees, TF8–5G9 completely inhibited thrombin generation but did not affect cytokine levels or fibrinolysis (118). In another study, TF8–5G9 significantly prolonged the median clotting time of plasma samples containing homogenized thrombi taken from STEMI patients (32).

The murine anti-human TF monoclonal antibody D3 also binds to the substrate interaction site of the TF:FVIIa complex (119, 120). D3H44 is a humanized form of the murine anti-human TF antibody D3 and binds to TF with a 100-fold increased affinity (121). It has been shown to significantly prolong clotting time of endotoxin-stimulated human whole blood and plasma (121, 122). An antibody that can act as an antidote, 6A6, has been developed and has been shown to completely reverse the effect of D3H44 (122).

The chimeric mouse anti-human TF antibody ch36 (ALT-836), also binds to the FX/FXII binding site of the TF:FVIIa complex (123). It reduced in-stent thrombosis in an in vitro study in which bare-metal stents were perfused with human blood (124). It was then evaluated in an open-label, dose-escalating trial that enrolled patients with stable coronary artery disease treated with ASA (123). No major bleeding occurred and minor bleeding occurred in a dose-dependent fashion (123). Whole blood clotting time was increased after treatment with ch36 (123). Another study...
showed that ch36 reduced platelet deposition after femoral artery injury in chimpanzees without a significant increase in bleeding (125). Most recently, it was demonstrated that ch36 can be safely administered to patients with sepsis-induced acute respiratory distress syndrome (126). However, no larger scale clinical trial has been performed to date.

Several drugs have been developed that interfere with the action of the TF:FVIIa complex. Three agents have been evaluated clinically. Recombinant nematode anticoagulant protein C (rNAPc2) is an anticoagulant protein that is derived from the hookworm Anclylostoma caninum (127). It binds to an exosite of FX/FXa and the active site of FVIIa and thereby inhibits the action of the ternary TF:FVIIa:FXa complex (127, 128). After subcutaneous injection it has a long half-life of approximately 50 hours (129). A phase II clinical trial investigating deep vein thrombosis prophylaxis in patients undergoing elective knee replacement showed that rNAPc2 had similar safety and efficacy to low-molecular-weight heparin (130). In two other phase II trials, rNAPc2 was found safe to administer together with standard therapy during elective coronary angioplasty (131) and after NSTEMI (132). However, no phase III trials have been performed to date.

An inactivated form of human FVIIa (FVIIai), which competes with FVII/FVIIa binding to TF, was also investigated in clinical trials. In a phase II trial of patients undergoing PCI, patients received FVIIai or placebo in addition to heparin (133). There was no difference between the two groups in the primary endpoint, which was a composite of death, MI, need for urgent revascularization, abrupt vessel closure or bailout with a GPIIb/IIIa antagonist or heparin at day 7 or hospital discharge. Rates of major bleeding were similar as well. Another phase II trial investigating the effect of single and multiple dosing strategies of FVIIai versus placebo in mechanically ventilated patients with acute respiratory distress syndrome was discontinued prematurely due to significantly higher 28-day mortality in the multiple dosing cohort receiving FVIIai (134). Overall, there was no significant difference but a trend to higher mortality and bleeding in patients treated with FVIIai compared to placebo (134). Due to these results, no further studies have been performed.

A recombinant form of TFPI (rTFPI) was found to be well tolerated in healthy volunteers (135). The results of a phase II study of rTFPI compared to placebo in patients with severe sepsis were promising as they showed 20% relative risk reduction of 28-day mortality, although there was an increase in major bleeding (9% vs. 6%) (136). However, a double-blind, randomized, phase III trial in patients with severe sepsis did not show any improvement in 28-day mortality and a higher risk of major bleeding (6% vs 4.8%) (137). Because post-hoc analysis showed that this result could have been caused by concomitant heparin administration in some patients, a second phase III trial in patients with severe community-acquired pneumonia was conducted which showed no difference in 28-day mortality and bleeding risk, despite evidence of biological activity (138). Due to these disappointing results, no further studies have been conducted.

In summary, despite some encouraging results with different TF:FVIIa inhibitors, major concerns with bleeding have limited their development. Ideally, one would like to inhibit pathological “thrombotic” TF expression without also inhibiting protective “haemostatic” TF and increasing haemorrhage. Statins represent an example of a drug that can inhibit pathological monocytes TF expression in hypercholesterolaemia without affecting vessel wall TF.

**TF and cardiac ischaemia/reperfusion injury**

The heart can be damaged during the ischaemia period of a MI and by the subsequent reperfusion. There is experimental evidence that activation of TF contributes to cardiac ischaemia/reperfusion injury (I/R) injury. In rabbits subjected to cardiac I/R, a marked increase in plasma TF activity was associated with a decrease in coronary flow (139). Consistent with these finding, we showed in a model of cardiac I/R injury in rabbits that inhibition of TF with an anti-TF antibody reduced infarct size by about 60% and also reduced chemokine expression and myocardial leukocyte infiltration (140). Similarly, inhibition of the TF:FVIIa complex with FVIIai resulted in a 50% reduction in infarct size (141). Inhibition of the TF:FVIIa complex on the membranes of apoptotic cells in rats after cardiac I/R injury also reduced infarct size significantly and attenuated the inflammatory response (142). Together, these findings indicate that TF contributes to cardiac I/R injury. This effect appears to be mediated through downstream signaling mediated by a fibrin degradation product (143) and protease-activated receptors (PARs).

There are four PARs, PAR1–4. They are expressed in the heart and cardiovascular system by cardiomyocytes, cardiac fibroblasts, endothelial cells and smooth muscle cells (1, 144). TF activates the coagulation cascade and generates thrombin, which activates PAR-1, 3 and 4 (145). PAR-1 can also be activated by the TF:FVIIa:FXa complex and MMP13 (146, 147). PAR-2 is activated by several proteases, including FXa (148, 149). Thrombin can also activate PAR-2 by binding to PAR-1 in a PAR1/PAR-2 heterodimer complex (149–151).

We found that PAR-1 deficiency does not influence infarct size after cardiac I/R injury (152). However, another group reported that a synthetic PAR-1 antagonist reduces infarct size after cardiac I/R injury in rats (153). These discordant results could be explained by possible off-target effects of the PAR-1 antagonist (154, 155). PAR-1 also appears to contribute to the worsening of cardiac function by inducing hypertrophic growth and morphological changes of cardiomyocytes (156, 157), and by inducing hypertrophy and pathological remodelling two weeks after cardiac I/R injury in vivo (152).

We found that a deficiency of PAR-2 is associated with decreased infarct size, oxidative stress and inflammatory cytokines after cardiac I/R injury in mice (158). PAR-2 deficient mice showed less impairment in heart function and dilatation of the left ventricle four weeks after cardiac I/R injury (158). These data indicate that PAR-2 contributes to pathological cardiac remodelling. The observed effects on pa-
thologic remodelling were independent of the effect of PAR-2 on infarct size and may be mediated by the pro-fibrotic chemokine MCP-1 (159).

In summary, TF-dependent thrombin generation and subsequent activation of PAR-1 as well as TF:FVIIa:FXa activation of PAR-2 contribute to hypertrophy and pathological remodelling after cardiac I/R injury in animal models. Based on these studies one would expect therapies that reduce TF expression or inhibit downstream targets, such as FXa, thrombin, PAR1, or PAR-2, would reduce pathological remodelling. Interestingly, therapies that have been shown to reduce pathological remodelling in humans, such as ACE inhibitors (87), beta-blockers (160) and mineralocorticoid receptor antagonists (161), also reduce TF expression (73, 99, 162). Anticoagulation with warfarin as a means to decrease thrombogenicity in heart failure on the other hand does not significantly improve outcomes (163). This could be due to the broader interaction of warfarin with the coagulation cascade (19). Currently, the ability of the FXa inhibitor rivaroxaban to decrease death, MI and stroke in patients with coronary artery disease and heart failure is under investigation (COMMANDER-HF). This could be in part achieved by reducing cardiac remodelling in addition to reducing increased thrombogenicity in patients with coronary artery disease and heart failure.

TF and heart failure

Heart failure is a clinical syndrome defined as the inability of the heart to meet the metabolic needs of the tissues. It arises in response to insults to the heart, such as myocardial ischaemia, volume overload, pressure overload, infiltrative or inflammatory diseases (164–166). These stimuli can cause the heart to undergo extensive remodelling that involves both cardiomyocytes and cardiac fibroblasts (164–166). Initially, the remodelling is an adaptive response to maintain cardiac function but in later stages the remodelling leads to a decreased heart function and eventually heart failure. The major processes contributing to pathological heart remodelling are cardiomyocyte hypertrophy, cardiomyocyte loss, proliferation of cardiac fibroblasts and cardiac inflammation (164, 166). Heart failure leads to extensive neuro-hormonal changes including an upregulation of the renin-angiotensin-aldosterone and sympathetic-adrenergic system. Further pathophysiological changes include a pro-inflammatory and pro-coagulant state along with platelet activation (167). These changes result in an increased risk of arterial and venous thromboembolic events (163, 168).

In addition to signalling through PARs, TF could have additional roles in more advanced heart failure. Reduced levels of TF are found in samples of the left ventricular myocardium in male patients that are over 60 years old, patients with hypertension and patients with left ventricular hypertrophy (169). TF mRNA and protein are also down-regulated in cardiomyocytes in myocardial biopsies that were obtained from patients with dilated cardiomyopathy, which is the most common end-stage of heart failure progression (170). One study showed detectable plasma TF activity in 40% of patients with systolic heart failure (171), although the source of TF was not defined. A shift in TF localization from the sarcolemma and Z-bands to the cytosol was observed when comparing patients with normal left ventricular ejection fraction to patients with reduced left ventricular ejection fraction (170). These findings suggest that TF could play a role in maintaining the structural integrity of the myocardium (172–174).

TF and viral myocarditis

Myocarditis is the inflammation of the myocardium in response to various agents, such as toxins, autoimmune disease, and infections. We analyzed the role of TF in a mouse model of viral Coxackievirus B3 (CVB3)-induced myocarditis (175). There are three main phases of viral myocarditis. In the early phase the virus infects cells within the heart and replicates causing the activation of the innate immune system. The acute phase is characterized by a declining virus load but increasing myocardial inflammation. In the late stage the virus is cleared and the inflammation recedes but pathological cardiac remodelling causes dilated cardiomyopathy and heart failure. CVB3 infection induces TF expression in the heart (176, 177), primarily during the early phase, and is associated with an increased thrombosis risk in the atria (178). The association between myocarditis and atrial thrombi was also observed in human subjects with left atrial thrombi due to atrial fibrillation (179). Interestingly, areas of myocarditis were close to the thrombus (179).

Importantly, we found that CVB3-infected PAR-1 deficient mice exhibited reduced levels of interferon (IFN)-β in the early phase of infection and increased viral loads and cardiac injury in the acute phase (180). Consequently, these mice showed increased cardiac remodelling and a lower cardiac function in the late stage of infection compared to wild-type mice (180). Similarly, inhibition of either TF or thrombin also increased CVB3 virus load in the heart and cardiac injury in wild-type mice (180).

Taken together these data suggest that the TF/thrombin/PAR-1 pathway enhances IFN-β expression and the innate immune response in CVB3 infected mice (180). These data suggest that anticoagulants or PAR-1 inhibitors may impair the innate immune response to some viruses.

Conclusion

TF has both protective and pathological roles in the heart. In the physiologic state it plays a crucial role in maintaining haemostasis in a mechanically highly active and vital organ.

In addition, in viral myocarditis TF appears to be protective by enhancing PAR-1 signalling and the innate immune response.

Therefore, inhibition of TF or downstream coagulation proteases or PAR-1 in this setting could be detrimental. This is especially important since anticoagulation therapy is used to prevent thrombosis in patients with atrial fibrillation and other prothrombotic conditions. These patients could be at in-
creased risk of adverse outcomes after viral infections.

The pathological roles of TF vary depending on the disease. In acute myocardial ischaemia the TF:FVIIa complex likely contributes to atherothrombosis. High levels of TF are present in atherosclerotic plaques and within the blood in the form of MPs. Risk factors for acute myocardial ischaemia are associated with increased levels of TF in the plasma. In contrast, therapies that lower the risk of cardiovascular events and mortality after acute myocardial ischaemia, such as statins and ACE inhibitors, decrease levels of TF in the plasma.

In addition, anticoagulants, such as rivaroxaban, are likely beneficial during thrombotic events, such as MI. For example, in the TIMI-51 trial FXa inhibition reduced mortality after MI. However, these benefits are associated with an increased bleeding risk. A possible solution could be inhibition of the intrinsic pathway instead of the common coagulation pathway as several animal studies show promising results by blocking FXI or FXII (50).

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Conflict of interest
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M. F. Bode; N. Mackman: Roles of tissue factor in the heart
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