# Tissue Factor in the myocardium: Evidence of roles in haemostasis and inflammation

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**Abstract**. The interaction between cell-surface tissue factor (TF) and the plasma coagulation factor VII (FVII) initiates the coagulation network that leads to the generation of thrombin and the formation of a fibrin clot. Thrombin also activates cellular protease activated receptors (PARs) through which it activates components of the inflammatory pathway. TF is expressed constitutively by cardiomyocytes and evidence from mice transgenic for a human TF mini-gene that express very low levels of human TF suggests that the TF-FVII interaction is critical for haemostasis within the heart. Pathological contact between TF and FVII may occur in the heart during ischaemia-reperfusion (I-R) injury and this may lead to activation of coagulation and thrombin generation. Evidence from animal models now suggests that thrombin is an important mediator of inflammation in I-R injury. The coagulation pathway therefore represents a novel therapeutic target for intervention in the prevention of I-R injury.

Keywords: Tissue factor, blood coagulation, inflammation, cardiomyocytes, ischaemia-reperfusion injury

## 1. Introduction

The transmembrane glycoprotein tissue factor (TF) is the cellular receptor and co-factor for the plasma coagulation factor VII (FVII) and the activated form of FVII, termed FVIIa. Binding of FVII to membrane bound TF leads to activation of FVII to FVIIa and the resulting TF/FVIIa complex activates coagulation factors IX and X (FIX and FX). In the absence of its co-factor, activated factor V (FVa), FXa generates only trace amounts of thrombin from prothrombin. The thrombin formed in this initiation stage of coagulation is insufficient to cause significant fibrin polymerisation but instead, back-activates the co-factors FV and factor VIII (FVIII). In this amplification stage of coagulation, FVIIIa forms a complex with FIXa and activates increasing quantities of FX. In turn, FXa forms a complex with FVa, and generates sufficiently large quantities of

thrombin to allow fibrin clot formation (Fig. 1). Although thrombin is the protease directly responsible for the generation of a fibrin clot, the interaction between TF and FVII is the initiating event in the coagulation pathway and is an important site at which haemostasis is regulated [1].

Inappropriate activation of coagulation is prevented in living organisms by maintaining an anatomical separation between TF on the surface of cells and FVII in the plasma. TF is expressed constitutively at high levels by cells of the vascular adventitia and in tissues that delimit organ and body boundaries [2]. In contrast, cells within the intravascular space normally show absent or very low level TF expression. Disruption of the endothelial lining of blood vessels may result in contact between TF and FVII and so may lead to activation of the coagulation pathway and the generation of thrombin [3]. The importance of avoiding inappropriate contact between TF and FVII is illustrated by pathological events such as disseminated intravascular coagulation in patients with sepsis from gram-negative bacteria. In this situation, coagulation may be activated if TF expression is induced on monocytes by bacte-

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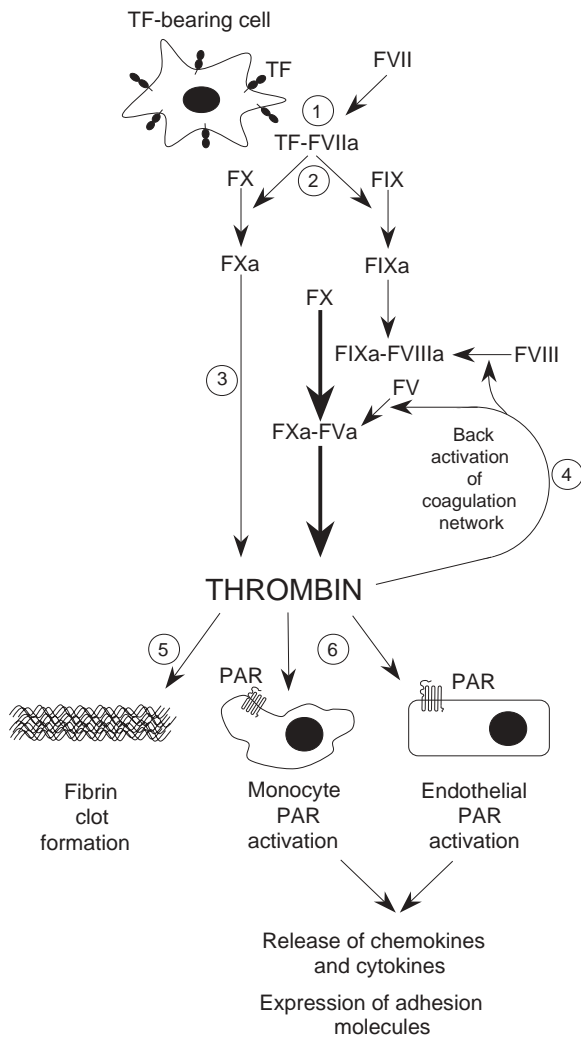
Fig. 1. The coagulation pathway is initiated by exposure of coagulation factor VII (FVII) to cells that express the integral membrane protein tissue factor (TF; 1). Activation of FVII to the active protease FVIIa in association with TF on the cell surface activates coagulation factor X (FX) to form FXa (2) which in turn generates the active protease thrombin (3). In most tissues, the small quantities of thrombin generated early in the coagulation pathway are insufficient to generate a fibrin clot. Instead, thrombin back-activates factors VIII and V (FVIII and FV) which then serve as cofactors to activated factor IX (FIXa) and FXa respectively, (4). This leads to greatly increased generation of FXa and thrombin. Only then can thrombin cleave fibrinogen to form fibrin (5) and activate inflammatory pathways by activating protease-activated receptors (PARs) on target cells such as monocytes and endothelium (6). Expression of large quantities of TF in myocardium may enable sufficient thrombin generation to enable fibrin formation and activation of inflammatory pathways without requiring back-activation of FV and FVIII.

rial lippopolysaccharide [4]. Similarly, coronary artery thrombosis causing myocardial infarction or unstable angina may follow the exposure of TF on activated

macrophages within atheromatous plaques to FVII in plasma after plaque rupture [5].

In addition to its well-established role as the initiator of coagulation, TF has now also been implicated as a mediator in the inflammatory pathway. Important evidence for this comes from observations that *in vitro*, pro-inflammatory stimuli up-regulate expression of TF on cells of the vasculature such as endothelium or monocytes [6]. TF is itself an important stimulus of the inflammatory response in disorders such as gram-negative sepsis, connective tissue disease, glomerulonephritis or the systemic inflammatory response to trauma [7–10].

Although, there is some evidence of a direct signalling role for the TF molecule itself [11], many of the pro-inflammatory activities of TF appear to be mediated by a group of cell-surface receptors termed the protease-activated receptors (PARs). PARs are a class of G-protein-coupled receptors that are activated through limited cleavage by a protease so that a new amino terminus is exposed. The new amino-terminus then acts as a tethered ligand that induces signalling through intramolecular binding [12]. Of the 4 known human members of this receptor class, PAR-1, PAR-3 and PAR-4 are activated by thrombin [12–14] and PAR-2 by trypsin, FVIIa and FXa [15]. PAR-1 is also activated by activated protein C [16]. Although PAR-1 and PAR-2 stimulate the expression of a wide range of common genes in vascular cells, there is not complete concordance. For example, the mRNA for monocyte chemoattractant protein (MCP-1) is induced by stimulation of PAR-1 but not PAR-2 [16].

The dominant PAR ligand in the human inflammatory response appears to be thrombin and through the PARs, thrombin activates endothelium and monocytes to express adhesion molecules and to release cytokines and chemokines [15,17] (Fig. 1). The coagulation and inflammatory pathways are therefore closely linked and share the common features that they may be initiated by the interaction of TF and FVII and that the effector protease is thrombin. Although both pathways require the generation of thrombin, the pro-inflammatory actions of thrombin do not require the generation of fibrin. Similarly, the pro-coagulant activity of thrombin on fibrin generation does not require the presence of PARs.

## 2. What is the pattern of tissue factor expression in the myocardium?

TF is expressed on cells of the cardiovascular system during the early stages of organogenesis in both

human and murine development. However, by embryonic day 9.5 in mice and at a comparable developmental stage in human embryos, TF protein and mRNA is demonstrable in the primitive heart [18]. In fully developed hearts, TF is expressed on adventitial cells of the coronary vasculature as in other tissues. However, cardiomyocytes also show high levels of constitutive expression of both antigenic and functional TF [2,4,19]. TF is expressed at greatest levels by contractile cardiomyocytes of the ventricles. Cardiomyocytes from the atria and cells of the cardiac electrical conducting system show lower level expression [20]. At ultrastructural level, TF is distributed on the cardiomyocyte sarcolemma but is concentrated at the intercalated discs between adjacent cardiomyocytes [2,20]. Since the intercalated discs contain the fasciae adherents into which the actin cytoskeleton inserts [21], this raises the possibility that TF may contribute to the structural integrity of the cardiomyocytes [20]. A structural association between TF and actin binding protein has previously been demonstrated in epithelial cells [22,23].

## 3. What are the effects of low-level tissue factor expression in the heart?

In transgenic mice in which the TF gene has been disrupted, approximately 90% of the TF-null embryos die by 10.5 days post-coitum from abnormal bleeding or developmental abnormalities in the vasculature [24–26]. However, if these mice are further manipulated to express human TF at very low levels by insertion of a TF 'mini-gene', most survive until term [27]. After birth, these 'low-TF' mice show a shortened lifespan in part because of an increased bleeding tendency at sites such as brain, lung and gastrointestinal tract. However, a consistent additional abnormality in these mice is haemosiderin deposition and fibrosis in the myocardium consistent with recurrent intra-cardiac bleeding [28]. The same changes are found in mice genetically modified to express low levels of FVII, but not in those that express low levels of coagulation FIX [29].

FIX and other components of the amplification stage of the coagulation pathway are vital for haemostasis in most tissues since they form an important back-activation loop by which thrombin generation is enhanced (Fig. 1). However, computer-generated and *in vitro* models of blood coagulation suggest that at very high concentrations of TF, the TF/FVIIa complex can generate enough thrombin for fibrin clot formation without needing amplification through the activation of the co-factors FV and FVIII [30]. This raises the interesting possibility that the high levels of TF in the myocardium are essential for cardiac haemostasis by providing a direct means of generating high levels of thrombin. This mechanism may be disrupted in both low-TF and low-FVII mice and consequently, these strains suffer intra-cardiac bleeds [28]. It is not yet clear whether data from other 'low-coagulation factor' mouse models will support this hypothesis [31].

## 4. Does myocardial tissue factor contribute to inflammatory pathways?

The ability of TF to initiate both coagulation and inflammatory pathways in other tissues raises the possibility that TF expressed in the myocardium may also have a dual role. One circumstance in which inflammatory pathways are activated in the myocardium is following restoration of blood flow after cardiac ischaemia. This common clinical scenario is termed myocardial ischaemia-reperfusion (I-R) injury and its consequences may range clinically from transient dysfunction of myocardial contractility to myocardial infarction [32]. These events may be a source of significant morbidity after interventions such as pharmacological thrombolysis for coronary artery thrombosis, coronary artery bypass surgery or angioplasty [33]. The pathophysiological events that underlie myocardial I-R injury include activation of monocytes, neutrophils and endothelium within the coronary circulation leading to the expression of adhesion molecules and the local release of pro-inflammatory cytokines and chemokines [34–37]. I-R injury may itself cause ischaemia and even infarction of the myocardium through plugging of the coronary microvasculature with aggregates of activated leucocytes [38].

As the pathophysiological events that initiate myocardial I-R injury have become clearer, TF has emerged as a potential mediator of inflammation. For example, oxygen free radicals are generated in the coronary circulation during reperfusion of ischaemic myocardium [39]. Oxygen free radicals also induce TF expression in cultured rabbit coronary endothelial cells [40]. TF pro-coagulant activity has been shown to increase in the coronary circulations of isolated rabbit hearts reperfused after ischaemia and this increase could be abolished by pre-treating the myocardium with oxygen radical scavengers [40]. In an open-chest rabbit model of myocardial I-R injury, reperfusion after only 5 minutes of ischaemia was also associated with

increased TF activity in the coronary vessels. The I-R injury in this model was not associated with ultrastructural changes in the coronary vasculature [39] so the likely source of this increased TF activity was the coronary endothelium rather than the cardiomyocytes [40]. Importantly, myocardial blood flow in the ischaemic area of the rabbit hearts during reperfusion could be improved by administration of a blocking anti-TF antibody [40]. A similar therapeutic benefit was achieved with human FVII that had been modified so that it retained the ability to bind TF but could not activate downstream components of the coagulation pathway (active site inhibited FVII) [41,42].

In an alternative model of myocardial I-R injury, rabbit hearts reperfused after 45 minutes of ischaemia show regions of both infarcted and non-infarcted tissue within the ischaemic territory [43]. In this circumstance, no increase in endothelial TF expression could be demonstrated in the coronary microvasculature from the non-infarcted ischaemic region. Instead, cardiomyocytes, which already show constitutive expression of TF, showed increased TF mRNA and antigen [44]. In this I-R injury model, there was ultrastructural evidence of disruption of the coronary vascular endothelial barrier in the non-infarcted area and deposition of fibrin in the myocardium [44]. These findings suggest that I-R injury may also be associated with extravasation of clotting factors, activation of coagulation by TF on cardiomyocytes and extravascular generation of fibrin [44, 45].

The effects of pharmacologically manipulating different components of the coagulation pathway have also been assessed in rabbit hearts reperfused after 45 minutes of ischaemia. Inhibition of TF with a blocking anti-TF antibody and inhibition of thrombin with hirudin during reperfusion both reduced the extent of myocardial infarction within the ischaemic territory. Inhibition of thrombin with hirudin also reduced neutrophil infiltration and secretion of the chemokines interleukin-8 (IL-8) and MCP-1 [44]. Although depletion of fibrinogen with ancrod abolished fibrin deposition in the myocardium it did not reduce infarct size. These findings suggest that both TF and thrombin have a role in the pathogenesis of myocardial I-R injury. They also suggest that although myocardial fibrin deposition is a feature of I-R injury, the generation of fibrin is not necessary for its pathogenesis.

Thrombin is known to stimulate endothelial cells to secrete the chemoattractants IL-8 and MCP-1 [46, 47] and to express intercellular adhesion molecule-1 (ICAM-1) and P-selectin [48]. These are necessary for recruitment, adhesion and extravasation of neutrophils in the inflammatory pathway that are an important cause of ischaemia and infarction in I-R injury [49,50]. The finding that the secretion of IL-8 and MCP-1, the extent of neutrophil infiltration and the size of myocardial infarction could all be reduced by thrombin inhibition in the rabbit myocardial I-R injury model is persuasive evidence that thrombin is a key mediator. PAR-1 is expressed on coronary vascular endothelium and smooth muscle cells [47] and this receptor mediates the pro-inflammatory actions of thrombin in other inflammation models [51]. It is tempting to speculate that the PARs are also important in myocardial I-R injury. Preliminary data is now available that suggests that a transgenic mouse strain that does not express PAR-1 appears to be protected against myocardial I-R injury [45]. Further phenotypic characterisation of this strain and studies involving pharmacological inhibition of the PARs in other I-R injury models are required to clarify their role.

## 5. Conclusion

Constitutive expression of TF on cardiomyocytes suggests an important role for TF or TF-dependent pathways in cardiac function. Phenotypic evidence from 'low-TF' transgenic mice suggests that expression of high concentrations of TF appear to be essential for local haemostasis. Possibly because of the unique mechanical demands placed on the coronary microvasculature, the myocardium may require a specialised robust local haemostatic system. TF expression on cardiomyocytes may provide this by acting as a secondary haemostatic barrier outside the coronary vascular adventitia. However, although the high constitutive expression of TF on cardiomyocytes may be necessary for haemostasis, it may also render the myocardium vulnerable to inflammatory stimuli by permitting the rapid local generation of thrombin. The role of the PARs in mediating the cellular responses to thrombin in the myocardium remains unclear and requires further experimental clarification.

Although evidence for a role of TF-dependent thrombin generation in animal models of I-R injury is compelling, evidence of involvement in human disease is more limited [52]. It is already established that the therapeutic use of hirudin reduces cardiovascular death, new myocardial infarction and re-occlusion rates in patients undergoing pharmacological thrombolysis [53, 54]. This raises the exciting possibility that pharma-

cological manipulation of other parts of the common coagulation-inflammatory pathway may also offer therapeutic benefit.

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