Tissue Factor and Atherothrombosis

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Atherosclerosis is a progressive disease characterized by the accumulation of lipids in medium to large sized arteries. Atherothrombosis is a term used to describe formation of a thrombus after rupture of an atherosclerotic plaque. Thrombosis can lead to myocardial infarction and stroke. Risk factors for atherosclerosis include hyperlipidemia, diabetes, smoking and hypertension all of which increase tissue factor (TF) expression. High levels of TF are present in atherosclerotic plaques due to expression by macrophages and vascular smooth muscle cells and the presence of cell-derived TF-positive microvesicles (MVs). In addition, hyperlipidemia leads to the formation of oxidized LDL, which induces TF expression in circulating monocytes and the release of TF-positive MVs. The major source of TF that drives thrombosis after plaque rupture is TF within the plaque. However, TF in the blood on monocytes and MVs may also contribute to the thrombosis. Inhibition of the TF/factor VIIa complex is unlikely to be an effective strategy to reduce atherothrombosis due to the essential role of the complex in hemostasis. However, selective blockade of pathologic TF without affecting protective TF may be effective in reducing atherothrombosis. For instance, statins have been shown to reduce TF expression in the plaque and in circulating monocytes, which would be expected to reduce thrombosis. Further studies are needed to determine safe strategies to reduce pathologic TF expression and atherothrombosis.


Key words: Tissue factor, Atherosclerosis, Atherothrombosis, Microvesicles, Hyperlipidemia

Tissue Factor

Tissue factor (TF) is the transmembrane receptor for factor VII/VIIa (FVII/VIIa). The TF/FVIIa complex is the major cellular initiator of the blood coagulation cascade leading to thrombin generation, fibrin deposition and platelet activation. It is inhibited by the anticoagulant protein tissue factor pathway inhibitor (TFPI). TF is constitutively expressed by cells surrounding the vessel wall, including pericytes and adventitial fibroblasts. This led to the proposal that TF acts as a hemostatic "envelope." TF is also expressed in a tissue-specific manner with high levels in various tissues, such as the heart and lung. We suggested that this parenchymal TF provides additional hemostatic protection to vital organs. Importantly, an imbalance between TF/FVIIa and TFPI can lead to either hemorrhage or thrombosis. Indeed, a complete absence of TF in mouse embryos leads to death due to hemorrhage, whereas an absence of TFPI in embryos leads to death due to thrombosis. We rescued TF-/- embryos by expressing low levels of human TF from a transgene. The so-called low TF mice (mTF-/-, hTF+) express ~1% of the level of TF of wild-type mice. However, adult low TF mice have hemostatic defects in various tissues, including the heart and lung. Interestingly, thrombosis in TFPI-/- embryos can be rescued by reducing the level of TF (using low TF mice), and conversely fatal lung hemorrhage in adult low TF mice can be dose-dependently attenuated by decreasing TFPI.
Tissue Factor in Plasma

Microvesicles (MVs) (also called microparticles or extracellular vesicles) are sub-micron sized vesicles that are released by apoptotic cells and activated cells, such as monocytes. Although TF is essentially undetectable in the blood of healthy individuals, levels of circulating TF in the form of MVs are increased in a variety of pathologic states, including hyperlipidemia. MVs are easy to isolate from plasma and TF+ MVs may be a good biomarker of a pro-thrombotic state. We recently reported that activity-based assays are more sensitive and may be more reliable than antigen-based assays for measuring the low levels of TF in plasma samples.

Atherosclerosis

Atherosclerosis is a progressive disease characterized by the accumulation of lipids in medium to large arteries, such as coronary arteries. Hyperlipidemia, diabetes, smoking, and hypertension are all risk factors for atherosclerosis. During atherosclerosis, formation of atherosclerotic plaques in the vessel wall results in narrowing of the lumen of the artery. Low density lipoprotein (LDL)-cholesterol from the circulation infiltrates into the arterial wall due to endothelial dysfunction during the early stages of atherosclerosis. Trapped LDL particles then become progressively oxidized resulting in the modification of LDL into biologically active lipids and proteins termed oxidized LDL (oxLDL). OxLDL activates monocytes/macrophages leading to increased expression of inflammatory mediators that enhance endothelial dysfunction, which further increases monocyte infiltration, macrophage foam cell formation, and production of oxLDL. This vicious cycle lies at the center of atherosclerotic disease.

Atherothrombosis

Atherothrombosis is defined as a ruptured atherosclerotic plaque with a thrombus. Formation of an occlusive thrombus can lead to myocardial infarction and stroke. Atherosclerotic plaques are highly procoagulant due to the presence of TF as well as various platelet activators, such as collagen. Atherosclerosis and subsequent atherothrombosis is the leading cause of death in the world.

TF Expression in Human Atherosclerotic Plaques

High levels of TF are expressed in atherosclerotic plaques and are associated with both cellular (macrophages, vascular smooth muscle cells [VSMCs]) and acellular (MVs and foam cell-derived debris within the necrotic core) regions. Atherosclerotic plaques have 200-fold higher concentrations of leukocyte, VSMC, and endothelial cell-derived MVs compared with circulating blood, and more than 50% of the MVs isolated from human plaques are TF-positive. Mallat and colleagues reported that 97% of the total procoagulant activity extracted from atherosclerotic plaques was due to TF. Subsequent proteomic analyses demonstrated that over 90% of MVs within plaques are CD14 positive, suggesting monocyte/macrophage origin. TF expression increases with the progression of the lesions. In addition, higher levels of TF activity are observed in coronary atheroma from patients with unstable angina compared to patients with stable angina, and in plaques with thrombi. Furthermore, lipid-rich plaques promote more clotting than less advanced lesions in an in vitro system. Taken together, these results support the notion that TF plays a key role in the formation of occlusive thrombi after plaque rupture.

TF Expression in Animal Models of Atherosclerosis

Various animal models of atherosclerosis have been developed to advance the understanding of how atherosclerotic plaques develop. The prominent models include rabbits, mice, swine, and non-human primates. Although animal models do not completely recapitulate human atherosclerosis, mice and rabbits are the most commonly used small and large models, respectively, for basic research.

i/ Rabbit Model

The rabbit has been used in many research facilities as an animal model of cholesterol diet-induced atherosclerosis. Among all the rabbit strains, New Zealand rabbits are the most popular strain used for atherosclerosis research. TF expression in atherosclerotic lesions from the thoracic aorta of cholesterol-fed rabbits. Aikawa et al. also reported that TF mRNA, TF protein expression, and TF activity were increased in the rabbit aorta atheroma induced by balloon injury.
and cholesterol feeding for 4 months \(^{38}\). Importantly, they observed that TF expression was reduced by lowering the amount of lipid in the diet \(^{38}\).

### ii/ Mouse Model

Mouse models have several advantages, such as inbred genetic backgrounds, easy breeding, low cost of maintenance, and the availability of a variety of difference transgenic lines. However, the use of mice as atherosclerotic models was limited because inbred strains did not develop spontaneous atherosclerosis \(^{41}\). In 1992, the first mouse model for atherosclerosis was generated by inactivating the ApoE gene (\(\text{ApoE}^{-/-}\)), a ligand for lipoprotein receptors \(^{42-44}\). Another popular gene-deficient model mice was generated by disruption of LDL-receptor gene (\(\text{Ldlr}^{-/-}\)). However, unlike \(\text{ApoE}^{-/-}\) mice, \(\text{Ldlr}^{-/-}\) mice need to be fed a diet with high cholesterol to develop hypercholesterolemia and atherosclerosis \(^{45}\). Cholesterol levels in \(\text{Ldlr}^{-/-}\) mice increase approximately two fold when on a chow diet and 15 fold when on an atherogenic diet \(^{45}\). Both mouse models respond to an atherogenic diet by developing complex lesions in the aortic root and whole aorta. High levels of TF expression are observed in the atherosclerotic lesions in \(\text{ApoE}^{-/-}\) mice \(^{46, 47}\). One study showed increased photochemical-induced thrombosis in atherosclerotic carotid arteries of \(\text{ApoE}^{-/-}\) mice compared with healthy arteries \(^{48}\). Furthermore, decreasing TFPI levels by 50% was associated with increased procoagulant activity, presumably due to increased levels of TF activity \(^{48}\).

### Role of TF in Atherothrombosis

Spontaneous rupture of atherosclerotic lesions is rarely observed in animal models and therefore it is difficult to assess the role of the TF/FVIIa complex in atherothrombosis. To overcome this problem, Chi and colleagues induced rupture of plaques in rabbits by a second balloon angioplasty, which resulted in thrombus formation in the injured vessel segment after a brief period of stasis. Administration of an inhibitor of the TF/FVIIa complex (active site-inhibited FVIIa [FVIIai]) before inducing rupture decreased the size of the thrombus in a dose-dependent manner, which indicated that the TF/FVIIa complex plays a critical role in thrombus formation in this model \(^{40, 49, 50}\).

Similar to the rabbit model, atherosclerotic plaques in mice rarely rupture. The Heemskerk group used ultrasound to rupture atherosclerotic plaques in carotid arteries of \(\text{ApoE}^{-/-}\) mice as a model of acute atherothrombosis \(^{51}\). Formation of thrombi is visualized by monitoring the accumulation of labeled platelets using a 2-photon laser scanning microscopy. They observed local damage, collagen exposure, luminal thrombus formation as well as intra-plaque intrusion of erythrocytes and fibrin. Recently, it was shown that FVIIai reduced thrombosis after rupture of atherosclerotic plaques in carotid arteries of \(\text{ApoE}^{-/-}\) mice, which indicated a role of the TF/FVIIa complex in atherothrombosis in this model (Fig. 1) \(^{51}\).

### TF Expression by Circulating Monocytes and Microvesicles

In \(\text{vitro}\) studies have shown that oxLDL, chemically modified LDL, and aggregated LDL induce TF expression in human monocytes/macrophages and the release of TF\(^+\) MVs \(^{52-55}\). In patients with hyperlipidemia, elevated levels of TF expression are observed in monocytes and monocyte-derived TF\(^+\) MVs \(^{14, 54, 56-58}\). Furthermore, patients with type II familial hypercholesterolemia have elevated levels of monocyte TF and TF\(^+\) MVs \(^{54, 56, 57}\). We recently reported that hypercholesterolemia in both mice and monkeys results in a step-wise increase in levels of oxLDL, TF expression in white blood cells, and MV TF activity, which was associated with activation of coagulation \(^{54}\). Activation of coagulation was reduced by administration of an anti-TF antibody and by genetically decreasing TF expression in hematopoietic cells \(^{59}\). Taken together, these findings support the notion that in addition to TF within the atherosclerotic plaque TF\(^+\) monocytes and monocyte-derived TF\(^+\) MVs may contribute to the formation of an occlusive thrombus after plaque rupture (Fig. 1).

### Statins and TF Expression

Statins inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which initiates the first step in cholesterol synthesis. Statins are used to lower cholesterol levels in hypercholesterolemic patients. In addition to lowering cholesterol levels, statins have been shown to possess other beneficial properties, such as anti-inflammatory activity and inhibition of prenylation of intracellular signaling proteins \(^{59-61}\). Importantly, statins reduce TF expression within atherosclerotic plaques in animal models \(^{46, 59, 62}\). Cerivastatin reduced TF expression in atherosclerotic lesions of hypercholesterolemic rabbits \(^{52}\). In \(\text{ApoE}^{-/-}\) mice, simvastatin and rosvastatin inhibit TF expression independent of plasma lipid levels, possibly by inhibition of Egr-1 and nuclear factor \(\kappa\)B (NF-\(\kappa\)B) \(^{46, 59}\). Another mechanism by which statins reduce TF expression is via inhibition of RhoA kinase.
Indeed, statins inhibit RhoA kinase activity in human aortic endothelial cells and monocytic cells\(^{(63, 64)}\). An additional possibility is that simvastatin activates the phosphatidylinositol Akt pathway, which has been shown to negatively regulate LPS induction of TF gene expression in monocytic cells\(^{(65)}\). Statins also reduce TF expression in monocytes in hypercholesterolemic patients and in animal models\(^{(53-57, 62, 66)}\). We found that simvastatin reduced plasma oxLDL in monkeys and mice in a lipid-independent manner\(^{(54)}\). Taken together, these findings suggest that statin can decrease TF expression and this may reduce the thrombogenicity of the atherosclerotic plaque and reduce TF expression in monocytes, which may lower the risk of forming occlusive thrombi after plaque rupture (Fig. 1).

**New Targets for Atherothrombosis**

The TF/FVIIa complex plays an essential role in hemostasis and therefore is not a good target for reducing atherothrombosis. Recently, there has been a growing interest in developing drugs that target the intrinsic/contact pathway of coagulation (factor XIIa [FXIIa], factor XIa [FXIa], and factor IXa [FIXa])\(^{(67)}\). This pathway amplifies the clotting cascade. Recently it was shown that either inhibition of FXIIa or a reduction in the level of FXI reduced atherothrombosis in a mouse model\(^{(68, 69)}\). Kuijpers \textit{et al.} showed that inhibition of FXIIa with either corn trypsin inhibitor or r-HA-infestin-4 did not affect the initial formation of the thrombus (which was dependent on the TF/FVIIa complex) but reduced the size of the thrombus\(^{(68)}\). Inhibition of FXIIa also increased embolization suggesting that this reduced the stability of the thrombus\(^{(68)}\). Similarly, van Montfoort \textit{et al.} found that lowering plasma FXI activity levels to 20% using an antisense oligonucleotide reduced thrombus formation 5 and 10 minutes after plaque rupture\(^{(69)}\). Inhibition of FXI also increased embolization suggesting that this reduced the stability of the thrombus\(^{(69)}\). Similarly, van Montfoort \textit{et al.} found that lowering plasma FXI activity levels to 20% using an antisense oligonucleotide reduced thrombus formation 5 and 10 minutes after plaque rupture\(^{(69)}\). Importantly, administration of the FXI antisense oligonucleotide did not affect the tail bleeding time. These findings are significant because thrombosis is reduced without an increase in bleeding\(^{(70)}\).

**Conclusion**

In humans as well as animal models, high levels of TF are present in atherosclerotic plaques. Hyperlipidemia is associated with a prothrombotic state, in part, by increasing TF expression on circulating monocytes and via the release of highly procoagulant TF\(^*\) MVs. Plaque-derived TF is likely to be a major trigger of atherothrombosis. In addition, TF in the blood in...
the form of monocyte TF and TF-positive MVs are also likely to contribute to thrombosis after plaque rupture. Targeting inducible, pathologic TF expression without affecting constitutive, hemostatic TF should be a safer strategy to reduce thrombosis in patients with atherosclerosis and cardiovascular disease.

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Competing Interests Statement

None.

References

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