New locations of intravascular tissue factor

Indications

C. Reinhardt
Vaskuläre Biologie und Hämostase, Institut für Klinische Chemie, Ludwig-Maximilians-Universität München, Germany

Keywords
Tissue factor, coagulation, encryption, activation, platelets, monocytes, eosinophils, neutrophils, microparticles, intravascular, blood-borne, inflammation

Summary
Tissue factor (TF) is the major initiator of blood coagulation, and a mediator of inflammation, angiogenesis, and carcinogenesis. According to recent evidence, preformed TF and inducible expression of the protein is observed in several blood components. TF is apparently constitutively expressed on circulating microparticles, and can be exposed within minutes on the cell membrane of activated eosinophils and platelets, and, potentially, on neutrophils. Expression in monocytes and neutrophils largely requires transcriptional activation of the TF gene. Eosinophils appear to harbour the highest concentration of preformed TF among all blood components under resting conditions. TF expression in eosinophil progenitor cells is substantially higher than in precursors of other granulocyte fractions. Eosinophil TF promotes transendothelial migration, which documents that presynthesized TF in blood supports functions apart from coagulation. It is still an open question how the intravascular TF is activated to trigger initiation of coagulation. TF activation in different blood components is likely to be differentially regulated according to the (patho)physiologic context.

Schlüsselwörter
Tissue-Faktor, Gewebethromboplastin, Blutgerinnung, Enkryptierung, Aktivierung, Plättchen, Monozyten, Eosinophile, Neutrophile, Mikropartikel, intravasal, Entzündung

Zusammenfassung
TF (tissue factor), ist das Starterprotein der Blutgerinnung. Außerdem ist TF bei Entzündungsprozessen, in der Gefäßbildung und bei der Entstehung von Tumoren von zentraler Bedeutung. Vor kurzem konnte TF in verschiedenen Blutbestandteilen nachgewiesen werden. TF wird auf zirkulierenden Mikropartikeln anscheinend konstitutiv exprimiert und kann innerhalb von Minuten auf der Zellmembran aktivierter Eosinophiler und Plättchen erscheinen. TF wird möglicherweise auch von Neutrophilen exprimiert. Die TF-Expression in Monozyten und Neutrophilen erfordert im wesentlichen die Transkription des TF-Gens. Eosinophile beinhalten scheinbar die höchste Konzentration an bereits gebildetem TF unter allen nicht aktivierten Blutbestandteilen. Die TF-Expression in eosinophilen Vorläuferzellen ist beträchtlich höher als in Vorläuferzellen anderer Granulozytenfraktionen. TF auf Eosinophilen fördert die transendotheliale Migration. Dies belegt, dass vorgebildeter TF im Blut noch weitere Funktionen außer der Gerinnung unterstützt. Auf welche Weise der intravasale TF aktiviert wird, um den Gerinnungsstart auszulösen, ist unklar. Die Aktivierung von TF in verschiedenen Blutbestandteilen wird höchstwahrscheinlich differenziell, entsprechend dem jeweiligen (patho)physiologischen Kontext, reguliert.

Hinweise für neue Lokalisierungen des intravasalen Tissue-Fakors

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Tissue factor (TF) is an integral membrane protein and a member of the type II cytokine receptor family. TF is constitutively expressed in cells of the adventitial and medial layers of the vessel wall and instantly initiates coagulation in the case of vascular injury. It binds and allosterically activates factor VIIa. The TF/VIIa complex binds its substrate factor X in the ternary initiator complex of coagulation (TF/VIIa/X). Factor Xa is generated leading to the formation of thrombin by the prothrombinase complex (Va/prothrombin/Xa) on the membrane of activated platelets. Thrombin is required for physiological hemostasis, since it cleaves fibrinogen to fibrin, which in turn forms polymers that prevent blood loss. It was found that TF synthesis can be induced in monocytes following proinflammatory stimuli. In vivo monocyte TF expression seems to be restricted to some pathologic situations, such as sepsis and atherothrombosis. However, platelets were recently found to store preformed TF, that is exposed on their surface after platelet activation. In addition, TF was detected on circulating microparticles that are both present in blood of healthy and diseased donors. It is still a matter of debate whether intravascular TF contributes to the pathogenesis of coagulation disorders.

Intravascular TF

In recent years the envelope paradigm of TF expression and function has been challenged by the demonstration of intravascular TF (blood-borne or circulating TF). From an in vitro thrombosis model, in which fresh human blood was perfused into a flow chamber to generate thrombi on collagen-coated slides it has been proposed that circulating TF contributes to the propagation of the growing thrombus (20). Independently, we observed that during a 5 minute stimulation of human blood with collagen type I, TF antigen appeared on the surface of platelets adhering to leukocytes (57). Most interestingly, this rapidly presented TF was able to start the coagulation cascade and its procoagulant activity was strongly increased in platelet-leukocyte conjugates. In addition, the presence of preformed TF on circulating microparticles was described in a study from the same laboratory (38). Subsequently, intravital fluorescence microscopy experiments on thrombus formation that was induced by a laser endothelial injury of a murine cremaster muscle arteriole showed that intravascular TF was concentrated at the thrombus-vessel wall interface (18, 19). In recent years several new cell types have been described to express TF.

Monocyte TF

TF de novo-synthesis in monocytes was first reported in 1975 by Rivers and co-workers observing procoagulant activity in endotoxin-stimulated leukocyte suspensions (49). TF expression on monocytes can be achieved by specific inflammatory stimuli, such as endotoxin (e. g. lipopolysaccharide) (21), phorbol esters (30), C-reactive protein (9) and proinflammatory mediators, like tumor necrosis factor a (TNF-α) (10) and interleukin 1-β (22). The TF and TNF-α genes are regulated by various transcription factors, including nuclear factor-kappa B/Rel proteins and Egr-1 (31). LPS-induced TF expression in human monocytic cells was found to be regulated by the transcription factors c-Fos/c-Jun, c-Rel/p65 and Sp1 (41). Interestingly, platelets were found to regulate monocyte TF activity. In 1974, Niemetz and Marcus (39) proposed that platelets enhance the procoagulant activity of white blood cells. This was also confirmed in monocyte cell cultures, in which isolated platelets added to monocytes enhanced LPS-induced TF activity (28, 46). Recently, in vitro interaction of endothelial microparticles with monocytes was shown to induce TF-dependent procoagulant activity (50). Increased expression levels of monocyte TF might play a role in sepsis (14, 29) and it was found that patients with unstable and stable coronary syndromes exhibit elevated levels of TF expression on circulating monocytes (26).

Monocyte TF in atherosclerosis

TF has been detected on monocyte-derived macrophages and macrophage foam cells that are associated with atherosclerotic lesions (25) and coronary plaques (24) indicating a role for TF in the development of arterial thrombi after plaque rupture. Inappropriate in vivo expression of monocyte TF is likely responsible for fibrin deposition in a variety of pathological conditions, such as sepsis-associated disseminated intravascular coagulation (DIC) (42) and thrombembolic disease (53).

Neutrophil TF

In rapidly processed blood (to avoid the activation of TF gene transcription), TF was barely noticeable in neutrophils by TF-specific ELISA measurements and no TF procoagulant activity could be detected (38). This observation is in accordance with the findings of Osterud and co-workers, who failed to detect TF antigen on neutrophils in stimulated whole blood (43). However, there is emerging evidence that neutrophils might be able to express TF under certain inflammatory conditions (34, 48).

Eosinophil TF

Blood eosinophils were found to store TF, which is mainly embodied in their specific granules and exposed on their cell membrane after cell activation (37). Eosinophils are the cells with the highest TF content in blood under resting conditions. They contain approximately one forth of the TF molecules compared to fully activated monocytes. Stimulation with GMCSF and PAF was accompanied by an increase in TF transcripts. Eosinophils selectively maintain a high TF expression during the haematopoiesis and consequently, part of the protein stored in mature eosinophils is likely to represent pre-synthesized TF derived from eosinophil progenitor cells. Our observations indicate TF as one of the critical mediators of the initial eosinophil migration across the activated endothelium. Eosinophil TF coagulant activity might be of relevance for the thrombogenesis promoted by hypereosinophilic conditions.

Platelet TF

There is strong evidence that platelets contain preformed TF, which is released within 5 minutes after collagen type I stimulation. Platelet TF contributes to the collagen triggered activation of blood coagulation (57). Immunoelectron microscopy revealed the presence of TF on the surface of platelets adhering to leukocytes. In unstimulated platelets, TF antigen was detected by double sandwich ELISA. Immunoelectron microscopy showed TF antigen localized in the α-granules and the open canalicular system of resting platelets. The ability of activated platelets to trigger the initiation of coagulation was low. This suggests that platelet TF is cryptic (36). One reason for the low TF procoagulant activity on activated platelets could be the concomitant release of tissue factor pathway inhibitor (TFPI), the physiologic inhibitor of the initiator complex of coagulation (16, 40). The presence of TF in platelets was confirmed by several authors (7, 17). It is still a matter of debate whether TF is transported to platelets by leukocyte-derived microparticles (11) and/or if the spliceosome of proplatelets that extend from megakaryocytes might potentially be capable of translating TF from pre-mRNAs (12, 52).

Endothelial TF

Endothelial cells isolated from human umbilical veins (HUVEC) generate TF activity following exposure to certain proinflammatory stimuli (8). However, TF mRNA and TF antigen was absent from endothelial cells lining normal internal mammary artery and saphenous vein samples (44, 56). Therefore, it is still an open question whether endothelial cells may express TF antigen in vivo.

TF of cell-derived microparticles

Microparticles are small membrane vesicles (<1 mm in diameter) that are released from the plasma membrane of cells upon activation (55), during apoptosis (2) and by shear stress (47). They constitute a heterogeneous population, differing in cellular origin, numbers, size, antigenic composition and functional properties. Microparticles are described to play a role in intercellular communication, immunity and coagulation (23). They support coagulation by exposure of negatively charged phospholipids that are essential for thrombin generation. In the case of monocyte- and platelet-derived microparticles their main, and probably central procoagulant function is the exposure of TF (13). Under physiologic conditions, 80% of the plasma microparticles are derived from platelets (6).

Most of the microparticles carrying TF express platelet-specific markers. In immunoblot analyses no TF antigen could be detected in circulating neutrophil-derived microparticles and in microparticles obtained from in vitro activated neutrophils, whereas TF was detected on platelet-derived microparticles and in vitro generated platelet microparticles (38). In the same study no TF antigen could be detected in neutrophil-derived microparticles by ELISA. Apparently, the filopodia of activated platelets are the preferential sites for the formation of TF-positive microparticles (37). In vitro generated monocyte-derived microparticles (51) and circulating monocyte-derived microparticles (19) were shown to expose TF on their membrane. Circulating monocyte microparticles are able to adhere to activated endothelial cells and to activated platelets by P-selectin / PSGL-1 interactions and interestingly they were found to play a significant role in fibrin stabilization of the nascent thrombus by the delivery of procoagulant TF. Monocyte microparticles were described to bind activated platelets or platelet-derived microparticles and facilitate fusion events between monocytes (or monocyte microparticles) and platelets (or platelet microparticles) leading to microparticles that are rich in active TF (45). On subpopulations of microparticles that were obtained from interleukin 1-α-stimulated human umbilical vein endothelial cells TF antigen could also be detected (1). The presence of microparticles has also been documented at sites of inflammation, such as the acellular lipid core of the atherosclerotic plaque (33). Furthermore, increased numbers of circulating microparticles have been reported in patients with acute coronary syndromes (32).

Activation of thrombogenic TF

Intravascular TF is in an inactive state when expressed on blood cells (encrypted or latent TF) (36). A discrepancy between TF antigen and the expression of TF procoagulant activity has been observed in a variety of cell types (35, 54). It was found that functional TF is entirely cell surface expressed on LPS-stimulated human blood monocytes and on a neoplastic cell line that expresses TF constitutively (15). Drake and co-workers also presented evidence that more than 90% of functional TF in cells is expressed on the cell surface and is fully competent for binding its ligand VII/VIIa. However, they found that the activity of TF on the intact cell surface is substantially lower than that in lysed cell preparations. A stimulus is required to express the latent proteolytic activity of the encrypted TF/VIIa complex (3, 4).

Until now a number of ways are known to induce cells to express encrypted TF procoagulant activity: freezing and thawing, sonication, protease treatment, phospholipase treatment, non-ionic detergents, apoptosis, complement, and Ca^{2+}-ionophores (5). There is a significant variation among these methods with respect to the level of TF procoagulant activity evoked as well as secondary effects on cell structure. Currently, the nature of TF de-encryption in blood is still unclear but it represents a major question in TF biology.

Conclusion

Murine arterial thrombosis models combined with intravital microscopy techniques recently led to a more detailed understanding of intravascular thrombus formation. These studies clearly indicate a major role of fibrin formation induced by microparticle TF in the stabilization of the nascent thrombus (18, 19). Despite this progress the role of intravascular TF in thrombus formation and inflammatory coagulation disorders is still unclear and further investigations are required to reveal the contribution of intravascular TF to thrombembolic pathologies.

Future studies should mainly concentrate on the role of microparticle-associated intravascular TF in thrombus formation, the detailed characterization of the cryptic state of intravascular TF and the underlying activation mechanisms of intravascular TF. Detailed knowledge on the sources of intravascular TF and its activation mechanisms might enable the development of therapeutic intervention strategies that may not be associated with an increased risk of bleeding complications.

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References

1. Abid Hussein MN, Meesters EW, Osmanovic N et al. Antigenic characterization of endothelial cell-derived microparticles and their detection ex vivo. J Thromb Haemost 2003; 1: 2434–43.
2. Aupeix K, Hugel B, Martin T et al. The significance of shed membrane particles during programmed cell death in vitro, and in vivo, in HIV-1 infection. J Clin Invest 1997; 99: 1546–54.
3. Bach RR, Moldow CF. Mechanism of tissue factor activation on HL-60 cells. Blood 1997; 89: 3270–6.
4. Bach RR, Rifkin DB. Expression of tissue factor procoagulant activity: regulation by cytosolic calcium. Proc Natl Acad Sci USA 1990; 87: 6995–9.
5. Bach RR. Tissue factor encryption. Arterioscler Thromb Vasc Biol 2006; 26: 456–61.
6. Berckmans RJ, Neiuwland R, Boing AN et al. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. Thromb Haemost 2001; 85: 639–46.
7. Camera M, Frigerio M, Toschi V et al. Platelet activation induces cell-surface immunoreactive tissue factor expression, which is modulated differently by antiplatelet drugs. Arterioscler Thromb Vasc Biol 2003; 23: 1690–6.
8. Camerer E, Kolsto AB, Prydz H. Cell biology of tissue factor, the principal initiator of coagulation. Thromb Res 1996; 81: 1–41.
9. Cermak J, Key NS, Bach RR et al. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood 1993; 82: 513–20.
10. Conkling PR, Greenberg CS, Weinberg JB. Tumor necrosis factor induces tissue factor-like activity in human leukemia cell line U937 and peripheral blood monocytes. Blood 1988; 72: 128–33.
11. Del Conde I, Shrimpton CN, Thiagarajan P et al. Tissue factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood 2005; 106: 1604–11.
12. Denis MM, Tolley ND, Bunting M et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. Cell 2005; 122: 379–91.
13. Diamant M, Tushuizen ME, Sturk A et al. Cellular microparticles: new players in the field of vascular disease? Eur J Clin Invest 2004; 34: 392–401.
14. Drake TA, Cheng J, Chang A et al. Expression of tissue factor, thrombomodulin, and E-selectin in

baboons with lethal Escherichia coli sepsis. Am J Pathol 1993; 142: 1458–70.
15. Drake TA, Ruf W, Morrissey JH et al. Functional tissue factor is entirely cell surface expressed on lipopolysaccharide-stimulated human blood monocytes and a constitutively tissue factor-producing neoplastic cell line. J Cell Biol 1989; 109: 389–95.
16. Engelmann B, Luther T, Muller I. Intravascular tissue factor pathway - a model for rapid initiation of coagulation within the blood vessel. Thromb Haemost 2003, 89: 3-8.
17. Engelmann B. Initiation of coagulation by tissue factor carriers in blood. Blood Cells Mol Dis 2006; 36: 188–90.
18. Falati S, Gross P, Merrill-Skoloff G et al. Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. Nat Med 2002; 8: 1175–81.
19. Falati S, Liu Q, Gross P et al. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. J Exp Med 2003; 197: 1585–98.
20. Giesen PL, Rauch U, Bohrmann B et al. Blood-borne tissue factor: Another view of thrombosis. Proc Natl Acad Sci USA 1999; 96: 2311–5.
21. Gregory SA, Morrissey JH, Edgington TS. Regulation of tissue factor gene expression in the monocyte procoagulant response to endotoxin. Mol Cell Biol 1989; 9: 2752–5.
22. Herbert JM, Savi P, Laplace MC et al. IL-4 inhibits LPS-, IL-1 beta- and TNF alpha-induced expression of tissue factor in endothelial cells and monocytes. FEBS Lett 1992; 310: 31–3.
23. Hugel B, Martinez MC, Kunzelmann C et al. Membrane microparticles: two sides of a coin. Physiology 2005; 20: 22–7.
24. Kaikita K, Ogawa H, Yasue H et al. Tissue factor expression on macrophages in coronary plaques in patients with unstable angina. Arterioscler Thromb Vasc Biol 1997; 17: 2232–7.
25. Landers SC, Gupta M, Lewis JC. Ultrastructural localization of tissue factor on monocyte-derived macrophages and macrophage foam cells associated with atherosclerotic lesions. Virchows Arch 1994; 425: 49–54.
26. Leatham EW, Bath PM, Tooze JA et al. Increased monocyte tissue factor expression in coronary disease. Br Heart J 1995; 73: 10–3.
27. Leon C, Alex M, Klocke A et al. Platelet ADP receptors contribute to the initiation of intravascular coagulation. Blood 2004; 103: 594–600.
28. Lorenzet R, Niemetz J, Marcus AJ et al. Enhancement of mononuclear procoagulant activity by platelet 12-hydroxyeicosatetraenoic acid. J Clin Invest 1986; 78: 418–23.
29. Lupu C, Westmuckett AD, Peer G et al. Tissue factor-dependent coagulation is preferentially upregulated within arterial branching areas in a baboon model of Escherichia coli sepsis. Am J Pathol 2005; 167: 1161–72.
30. Lyberg T, Prydz H. Phorbol esters induce synthesis of thromboplastin activity in human monocytes. Biochem J 1981; 194: 699–706.
31. Mackman N, Brand K, Edgington TS. Lipopolysaccharide-mediated transcriptional activation of the human tissue factor gene in THP-1 monocytic cells requires both activator protein 1 and nuclear factor kappa B binding sites. J Exp Med 1991; 174: 1517–26.
32. Mallat Z, Benamer H, Hugel B et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation 2000; 101: 841–3.
33. Mallat Z, Hugel B, Ohan J et al. Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques: a role for apoptosis in plaque thrombogenicity. Circulation 1999; 99: 348–53.
34. Maugeri N, Brambilla M, Camera M et al. Human polymorphonuclear leukocytes produce and express functional tissue factor upon stimulation. J Thromb Haemost 2006; 4: 1323–30.
35. Maynard JR, Dreyer BE, Stemerman MB et al. Tissue factor coagulant activity of cultured human endothelial and smooth muscle cells and fibroblasts. Blood 1977; 50: 387–96.
36. Maynard JR, Heckman CA, Pitlick FA et al. Association of tissue factor activity with the surface of cultured cells. J Clin Invest 1975; 55: 814.
37. Moosbauer C, Morgenstern E, Cuvelier SL et al. Eosinophils are a major intravascular location for tissue factor storage and exposure. Blood 2006; in press.
38. Muller I, Klocke A, Alex M et alIntravascular tissue factor initiates coagulation via circulating microvesicles and platelets. FASEB J 2003; 17: 476–8.
39. Niemetz J, Marcus AJ. The stimulatory effect of platelets and platelet membranes on the procoagulant activity of leukocytes. J Clin Invest 1974; 54: 1437–43.
40. Novotny WF, Girard TJ, Miletich JP et al. Platelets secrete a coagulation inhibitor functionally and antigenically similar to the lipoprotein associated coagulation inhibitor. Blood 1988; 72: 2020–5.
41. Oeth P, Parry GC, Mackman N. Regulation of the tissue factor gene in human monocytic cells. Role of AP-1, NF-kappa B/Rel, and SP1 proteins in uninduced and lipopolysaccharide-induced expression. Arterioscler Thromb Vasc Biol 1997; 17: 365–74.
42. Osterud B, Bjorklid E. The tissue factor pathway in disseminated intravascular coagulation. Semin Thromb Hemost 2001; 27: 605–17.
43. Osterud B, Rao LV, Olsen JO. Induction of tissue factor expression in whole blood: lack of evidence for the presence of tissue factor expression in granulocytes. Thromb Haeost 2000; 83: 861–7.
44. Osterud B, Tindall A, Brox JH et al. Thromboplastin content in the vessel walls of different arteries and organs of rabbits. Thromb Res 1986; 42: 323–9.
45. Osterud B. The role of platelets in decrypting monocyte tissue factor. Semin Hematol 2001; 38: 2–5.
46. Pinder PB, Hunt JA, Zacharski LR. In vitro stimulation of monocyte tissue factor activity by autologous platelets. Am J Hematol 1985; 19: 317–25.
47. Reininger AJ, Heijnen HF, Schumann H et al. Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. Blood 2006; 107: 3537–45.
48. Ritis K, Doumas M, Mastellos D et al. A novel c5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. J Immunol 2006; 177: 4794–802.
49. Rivers RP, Hathaway WE, Weston WL. The endotoxin-induced coagulant activity of human monocytes. Br J Haematol 1975; 30: 311–6.
50. Sabatier F, Roux V, Anfosso F et al. Interaction of endothelial microparticles with monocytic cells in vitro induces tissue factor-dependent procoagulant activity. Blood 2002; 99: 3962–70.
51. Satta N, Toti F, Bohbot A et al. Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharide. J Immunol 1994; 153: 3245–55.
52. Schwertz H, Tolley ND, Foulks JM et al. Signal-dependent splicing of tissue factor pre-mRNA modulated the thrombogenecity of human platelets. J Exp Med 2006, in press.
53. Semeraro N, Colucci M. Tissue factor in health and disease. Thromb Haemost 1997; 78: 759–64.
54. Walsh JD, Geczy CL. Discordant expression of tissue factor antigen and procoagulant activity on human monocytes activated with LPS and low dose cycloheximide. Thromb Haemost 1991; 66: 552–8.
55. Wiedmer T, Sims PJ. Participation of protein kinases in complement C5b-9-induced shedding of platelet plasma membrane vesicles. Blood 1991; 78: 2880–6.
56. Wilcox JN, Smith KM, Schwartz SM et al. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci USA 1989; 86: 2839–43.
57. Zillmann A, Luther T, Muller I et al. Platelet-associated tissue factor contributes to the collagen-triggered activation of blood coagulation. Biochem Biophys Res Commun 2001; 281: 603–9.

Correspondence to:
Christoph Reinhardt
Vaskuläre Biologie und Hämostase
Institut für Klinische Chemie, Ludwig-Maximilians-Universität
Marchioninistraße 15, 81377 München, Germany
E-mail: Christoph.Reinhardt@med.uni-muenchen.de