Endothelial cells, tissue factor and infectious diseases

Abstract

Tissue factor is a transmembrane procoagulant glycoprotein and a member of the cytokine receptor superfamily. It activates the extrinsic coagulation pathway, and induces the formation of a fibrin clot. Tissue factor is important for both normal homeostasis and the development of many thrombotic diseases. A wide variety of cells are able to synthesize and express tissue factor, including monocytes, granulocytes, platelets and endothelial cells. Tissue factor expression can be induced by cell surface components of pathogenic microorganisms, proinflammatory cytokines and membrane microparticles released from activated host cells. Tissue factor plays an important role in initiating thrombosis associated with inflammation during infection, sepsis, and organ transplant rejection. Recent findings suggest that tissue factor can also function as a receptor and thus may be important in cell signaling. The present minireview will focus on the role of tissue factor in the pathogenesis of septic shock, infectious endocarditis and invasive aspergillosis, as determined by both in vivo and in vitro models.

The structure and location of tissue factor

Tissue factor, also known as thromboplastin or CD142 (rarely as coagulation factor III), is a cell surface glycoprotein synthesized and expressed by a wide variety of cells (1-3). Tissue factor triggers the extrinsic coagulation pathway, plays a key role in homeostasis and in several thrombotic diseases, and is also involved in cell signaling (4-10). Tissue factor is a glycosylated membrane protein that consists of a single polypeptide chain of 263 amino acids with an apparent molecular mass of 46 kDa, as determined by SDS-PAGE electrophoresis (5,11-14). Glycosylation does not appear to be important for tissue factor function as the recombinant nonglycosylated protein retains procoagulant activity (12). Tissue factor is a type I integral membrane protein. It has three domains: an extracellular domain, a single transmembrane domain, and a short cytoplasmic domain. The binding site for factor VII is located in the extracellular domain (5,15).

Tissue factor is expressed by a diversity of cells such as smooth muscle cells, fibroblasts, monocytes, lymphocytes, granulocytes, platelets and endothelial cells (1,3). Smooth muscle cells and fibroblasts express...
tissue factor constitutively (1). Following tissue injury, these cells come in contact with the bloodstream, and rapidly initiate the coagulation cascade. Myeloid cells and endothelial cells only express tissue factor when they are stimulated (16-19). They can be activated by proinflammatory cytokines, bacterial lipopolysaccharide (LPS), and some microorganisms (16,20-22). The expression of active tissue factor on the cell surface requires at least 2 h in cultured cells (16). Activated cells can also release membrane microparticles into the circulation. These microparticles also contain tissue factor on their surface (7). However, the presence of tissue factor-containing microparticles in the circulation does not correlate with in vivo markers of coagulation, suggesting that tissue factor may play a role in another process, such as signaling or angiogenesis (23).

Tissue factor may also be present on the surface of endothelial cells, yet be inactive. This encrypted form of tissue factor is located mainly in caveolae of the endothelial cells (7). Encrypted tissue factor is capable of binding factor VIIa, but the complex remains catalytically inactive and incapable of initiating coagulation (24-26). Thus, encryption seems to prevent unwanted activation of intravascular thrombosis. The mechanism by which tissue factor is encrypted is still incompletely understood.

The extrinsic coagulation cascade

The initiation of coagulation following tissue injury requires the exposure of tissue factor to the bloodstream. Tissue factor then binds factor VII/factor VIIa that circulates in the blood (27). The tissue factor/factor VIIa complex activates both factor IX and factor X, thus leading to the sequential conversion of the blood clotting proenzymes to enzymes and the generation of a fibrin clot (Figure 1). The tissue factor or extrinsic pathway is linked to the intrinsic pathway by the activation of factor VIII and factor IX, which mediates thrombus propagation and extension. Thus, tissue factor plays a pivotal role in coagulation by initiating and propagating thrombus formation.

Anticoagulant properties of normal endothelium

Under normal conditions, endothelial cells prevent the activation of the coagulation cascade by expressing several surface molecules with anticoagulant properties (28). The most important homeostatic mechanisms that inhibit coagulation are: 1) the antithrombin III-heparin sulfate system, which inhibits thrombin and factor Xa; 2) tissue factor protease inhibitor, which is composed of three Kunitz-type protease inhibitor domains, and directly inhibits factor Xa and mediates a negative feedback on tissue factor expression and factor VIIa (29,30), and 3) the protein C pathway (31,32). The pro-
tein C pathway is activated when thrombin binds to the endothelial surface protein thrombomodulin (or CD141) and is facilitated by the transmembrane endothelial protein C receptor (33). In sepsis, endothelial damage and the presence of proinflammatory stimuli (e.g., TNF-α, endotoxin) down-regulate both thrombomodulin and endothelial protein C receptor, thereby promoting intravascular coagulation. The molecular links between inflammation and coagulation are unquestionable. Inflammation promotes coagulation by leading to intravascular tissue factor expression and down-regulation of the fibrinolytic and protein C anticoagulant pathways. Protein C and antithrombin III are quickly depleted in sepsis as the body attempts to reestablish equilibrium (6). In fact, the level of circulating protein C is inversely related to mortality in patients with severe sepsis.

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Septic shock is a major health problem. It is the leading cause of death in intensive care units. Early studies of septic shock focused on inflammation as the dominant process causing vascular endothelial injury and the so-called sepsis cascade. However, given the universal failure of specific anti-inflammatory therapies in clinical trials of septic patients, a search for a more complex and multifactorial pathogenesis was undertaken (34). The new paradigm that has emerged from those investigations, which has radically changed the view of sepsis, is the current understanding that the disease process is caused by a loss of homeostasis between inflammation, coagulation, and fibrinolysis (35-38). This new paradigm is supported by the recent finding that recombinant activated protein C significantly reduces mortality in patients with sepsis (39).

Bacterial LPS and the inflammatory cytokines, TNF-α and interleukin-1, have been shown to be important mediators of septic shock (40). One mechanism by which the proinflammatory mediators contribute to septic shock is by stimulating tissue factor expression, which in turn activates the coagulation cascade (37,41). In experimental animal models of gram-negative septic shock, a monoclonal antibody against tissue factor attenuates coagulopathy and protects against death (42).

Infectious endocarditis is characterized by the formation of valvular vegetations consisting of bacteria embedded within a platelet-fibrin meshwork. Tissue factor plays a key role in the development of these vegetations. From *in vitro* studies, it has been discovered that *Staphylococcus aureus* infection induces tissue factor synthesis and expression on endothelial cells and monocytes (43-45). While endothelial cells play an important role in the early events of vegetation formation, monocytes participate by intensifying fibrin deposition (44,46). There is some controversy about the importance of live or killed bacteria in tissue factor induction (45,47). Recently, it was demonstrated that the cell wall peptidoglycan of *S. aureus* induced tissue factor synthesis and expression on CD14-positive monocytes. The kinetics of tissue factor induction by LPS and peptidoglycan is similar, with maximal procoagulant activity developing within 4 h. However, LPS is a 10-fold more potent stimulus of tissue factor expression compared to peptidoglycan (47).

Although *S. aureus* is able to induce endothelial cell tissue factor-mediated procoagulant activity in endothelial cells, other bacteria that cause infectious endocarditis, such as *Streptococcus sanguis* and *Staphylococcus epidermitis*, cannot (45). With these organisms, it is hypothesized that the formation of the vegetation is induced by tissue factor expressed by monocytes, rather than the endothelial cells.

*Aspergillus fumigatus* is an angioinvasive fungus, and invasive aspergillosis is characterized by vascular invasion with subsequent...
thrombosis and tissue infarction (48). We have developed an in vitro model of interaction of A. fumigatus with human umbilical vein endothelial cells (Lopes-Bezerra LM and Filler SG, unpublished results). Our data indicate that A. fumigatus stimulates endothelial cells to express tissue factor and become procoagulant. Candida albicans is another angioinvasive fungus. However, vascular thrombosis is not usually seen at foci of candidal infection. Interestingly, we found that C. albicans did not induce endothelial cell tissue factor activity in vitro. Similarly, in a patient with candidemia, it was found that the monocytes did not express tissue factor. In contrast, monocytes from patients with systemic bacterial infections strongly expressed tissue factor (49). These observations suggest that tissue factor expression is tightly controlled and is induced only in response to specific microbial pathogens.

In conclusion, activation of endothelial cell tissue factor-mediated procoagulant activity is a key event in the pathogenesis of several types of infection. Understanding this process and developing methods to control it hold great promise for improving the outcome of these severe and often fatal infections.

References


