TISSUE FACTOR; FROM MORAWITZ TO MICROPARTICLES

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ABSTRACT

Tissue factor is the principal activator of blood coagulation *in vivo.* The existence of extravascular tissue factor has been recognized for over a century, but a rational role as a cell-based enzymatic cofactor in blood coagulation was first proposed by Paul Morawitz in 1905. By the close of the last century, very low levels of circulating tissue factor had been identified in the intravascular compartment, but its role in health and in hemostatic and thrombotic disorders continues to be debated. Nonetheless, ongoing research suggests that tissue factor may be a rational therapeutic target in a number of human disease states.

Introduction

Tissue factor (TF) is a 47kDa cell-bound trans-membrane glycoprotein that is constitutively expressed by many extravascular cells, including fibroblasts, pericytes, astrocytes, and cardiomyocytes (1). Certain organs, including the heart, brain, testis, placenta and kidney are particularly enriched in TF, whereas others, such as skeletal muscle and liver, are not (2,3). In the healthy vessel wall, neither the endothelial monolayer nor the cells comprising the underlying intima express any significant TF antigen (2,4). Membrane-bound TF binds either plasma FVII or FVIIa to, respectively, promote FVII activation or enhance catalytic activation of factors IX or X, ultimately leading to the generation of a cross-linked fibrin clot. In addition to its involvement in the initiation of coagulation, TF is now recognized to play a role in a range of biological processes. These include migration and proliferation of vascular smooth muscle cells (5), development of embryonic blood vessels (6), tumor neovascularization and metastasis (7), and induction of the pro-inflammatory response (8). However, this

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brief review will primarily focus on the best characterized of these activities, namely the initiation of physiological coagulation (hemostasis) and the initiation and propagation of pathological thrombus formation.

History of the Evolution of Blood Coagulation Paradigms

Otherwise known as (tissue) thromboplastin or coagulation factor III, TF derives its name from the fact—first discovered in the mid 19th century—that tissue extracts initiate coagulation upon contact with blood or plasma. In 1905, Paul Morawitz, while still at the University of Tubingen, coined the term "thrombokinase' to describe the clot-promoting principle in tissue and suggested that platelets were the principal source. Although also an early pioneer in Transfusion Medicine and vascular biology (9), Morawitz is best remembered for his "classic" theory of blood coagulation, which recognized 4 necessary and sufficient components for blood coagulation; thrombokinase, calcium, prothrombin, and fibrinogen (10).

Later in the 20th Century, as additional coagulation factors were discovered and characterized, it became clear that a more inclusive model of coagulation was needed to explain this aspect of physiology. In 1964, two groups on either side of the Atlantic presented an alternative model that was envisioned as a sequential "cascade' (11,12), comprised of two distinct physiological pathways capable of initiating coagulation—the "intrinsic' (contact) and the "extrinsic' (TF-dependent) pathways. Subsequently, TF apoprotein was purified to homogeneity (13), its cDNA isolated and sequenced (14,15) and the 3D-structure of the TF-VIIa complex described (16). The demonstration that the TF-VIIa complex activates not only factor X, but also factor IX (17), as well as the characterization of the natural inhibitor of the TF-VIIa complex—tissue factor pathway inhibitor (TFPI) (18)—and a better understanding of the role of factor XI in coagulation (19) collectively re-focused the paradigm on the TF-VIIa complex as the sole initiator of coagulation *in vivo.* In this revised model of coagulation, the "extrinsic' and "intrinsic' pathways were postulated to act in series to generate thrombin, as opposed to the cascade hypothesis in which the two pathways were envisioned as operating in a parallel fashion that converged on the "final common pathway' (20).

Intravascular TF

It has been known for more than 2 decades that *in vitro* activation of endothelial cells or monocytes by endotoxin, inflammatory cytokines

and immune complexes is associated with transcriptional induction of TF synthesis. In 1999, Nemerson's group demonstrated that normal blood contains circulating TF in the form of microparticles (21). At about the same time, our group described a novel assay for the quantitation of the very low levels of circulating TF procoagulant activity in whole blood (22). In retrospect, this assay was sensitive to both cell-associated and microparticle-associated TF in the circulation. Although still lacking a consensus definition, microparticles are generally defined as submicron-sized cell-derived membrane fragments produced in response to activation or apoptosis. It was subsequently demonstrated that blood-borne TF (in the form of microparticles) is needed for normal thrombus extension in a laser-induced microvascular injury model in mice (23). However, some controversy remains regarding the role of blood-borne TF in hemostasis, because as a trace protein, its measurement is a formidable challenge, and in murine models, the absolute requirement for blood-borne TF—as opposed to vessel wall TF—appears to be dependent on the model of vascular injury, as well as the size of the vessel that has been injured (24,25). However, many laboratories, including our own, have been able to demonstrate the presence of TF-bearing microparticles in blood using flow cytometry (26). The levels of TF-bearing microparticles appear to be increased in a variety of vascular and hematopoietic disorders, such as thrombotic stroke (27), anti-phospholipid antibody syndrome (28), cancer (29), and sickle cell disease (30).

There have been fewer studies focusing on the development of assays to measure circulating TF procoagulant activity. This is arguably a more important parameter, since it is well known that the relationship between TF antigen and activity in cell membranes may correlate poorly. The primary post-translational mechanism of regulation of TF activity is through a process of self-association and de-association that has been termed in the literature ''encryption' and ''decryption'. Encryption refers to the fact that TF may bind FVII/VIIa in a cell membrane, yet exhibit only a fraction (even 10% or less) of its full procoagulant activity. Part (but not all) of this masked activity is explained by the orientation of the membrane phospholipid headgroups in resting *vs.* activated cells (31,32).

We recently developed a relatively facile technique for capturing and measuring microparticle-associated TF activity in human cell-free plasma samples. Although working close to the limits of detection, we demonstrated approximately an 8-fold increase in activity in human volunteers within hours after exposure to endotoxin (33). Furthermore, higher levels of microparticle-associated TF procoagulant activity were

evident in individuals who were homozygous for an insertion polymorphism at nucleotide position—1208 in the promoter region (34). Additional studies examining the role of blood-borne microparticle- and cell-associated TF are underway in other disease states, including venous thrombosis in humans and baboon models, and in patients with breast cancer. One limitation of all available assays for TF is the lack of an accepted standard; fortunately, this need is currently being addressed by the Scientific and Standardization sub-committees of the International Society of Thrombosis and Haemostasis (35).

Tissue factor and cardiovascular disease

While TF antigen is immunocytochemically detectable only in adventitial cells of the normal arterial wall, it is present in endothelial and smooth muscle cells, foam cells and macrophages, as well as in the surrounding extracellular space (in the form of microparticles) located in the intima of the atherosclerotic plaque (4,36). A number of potentially relevant agonists, including platelet-derived growth factor (PDGF-BB) (37), oxidized LDL (38,39), and CD40 ligand (40), as well as C-reactive protein (CRP) (41) may be responsible for the induction of TF synthesis in these target cells (42). Similarly, endothelial infection by viral agents implicated in the pathogenesis of atherosclerosis may result in TF induction (43). Rupture of the cap of the plaque is thought to initiate thrombosis by exposing TF to circulating FVII(a); thus, strategies that inhibit TF procoagulant activity involve an important new class of anti-thrombotic agents that are currently in clinical trials in patients with coronary artery disease undergoing invasive procedures (42,44). Another potential area in which TF may represent a rational therapeutic target is in the prevention of arterial restenosis due to neointimal hyperplasia (42). We previously demonstrated that higher baseline levels of circulating whole blood tissue factor procoagulant activity may be a predictor of restenosis after percutaneous transluminal coronary angioplasty and stent implantation in patients with coronary atherosclerotic disease (45). In an animal model of arterial injury, inhibition of TF synthesis by a small molecular weight inhibitor of NF-κB dependent transcription significantly blunted restenosis, re-capitulating the effect of an inhibitory antibody to TF (46). This inhibitor also partially prevented venous thrombosis in an inferior vena cava ligation model. These and other recent observations support the contention that we are on the threshold of an era in which TF is viewed as a major therapeutic target, not only in the inhibition of arterial and venous thrombosis (47,48), but also in the containment

of the systemic inflammatory response (49,50), cancer progression (51,52), and atherosclerosis (53).

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DISCUSSION

Colwell, Charleston: I enjoyed it very much. I was thinking of type II diabetes, which is oftentimes called a prothrombotic or procoagulant state, we say of a number of thromboses. Has tissue factor activity been looked at in type II diabetes?

Key, Chapel Hill: Yes, actually I would say that being someone more interested in venous thrombosis, diabetes mellitus wasn't originally considered to be much of a risk factor. I think that view is changing with new data that support diabetes as a risk factor for venous thrombosis. Recently in fact, Dr Koneti Rao's group used our whole blood assay in a study in which they clamped insulin and blood sugar levels in volunteers and showed that both independently induced tissue factor expression in vivo. The study was published earlier this year in "Diabetes', I think.

Antman, Boston: We have been exploring the inhibition of tissue factor in some clinical trials in cardiology, and we have run into two issues that I am concerned about, which may narrow the therapeutic window for its use. One is that if you go up too high on the dose, you actually run into spontaneous bleeding or dry purpura is also seen. The second is despite the inhibition of tissue factor or other inhibition of the coagulation cascade quite proximally, it is very difficult to feel safe about using these drugs in the catheterization laboratory, because of reports of catheter-related thrombus. In fact, some of the factor Xa inhibitors now are contraindicated to be used alone in the cath lab. They have to be supported by drugs that give additional antithrombin action, like unfractionated heparin. Any comments about this?

Key: Which tissue factor inhibitors are you using?

Antman: Well both. NAP-c2 and cH36.

Key: Okay. So, more than 10 years ago Harker's group at Emory suggested that tissue factor could be the holy grail of anticoagulation, because if we had relatively little tissue factor that needed inhibiting in the intravascular compartment or on the endothelium, and a lot of protective tissue factor around the vessel wall, that the therapeutic index, which is the limitation, of course, of all anticoagulants, could be increased by using a tissue factor inhibitor. What your data, and others, is suggesting is that is probably not the case in terms of bleeding risk. And I don't really understand the reasons why. I think that we still have a lot to learn about the whole encryption phenomenon of tissue factor, and what form we are going after, for example. I am interested you see dry purpura which I also can't explain. That's an odd manifestation for an anticoagulant and suggests that you are seeing inhibition of a primary hemostatic phase. I don't really understand the mechanism at this point.

King, Atlanta: You mentioned the effect on restenosis of the studies you have done showing circulating tissue factor increase, that in stent placement now restenosis has become much less of a problem with drug-eluting stents, but late thombosis of those stents a long time after they are placed has arisen as the major concern. Cy Wilcox has shown recently that nitric oxide production in endothelial cells is abnormal in the drug-eluting stents. I wonder if you have done any work with tissue factor, would you project that this may be abnormal, and if so, can you have any speculation about why endothelial cells affected by such agents would retain that effect long after the agent is gone?

Key: Well, I knew I'd get into trouble by showing cardiology data at this meeting, but I think the argument is going to come down to the location. Is the tissue factor originating from the blood? Depending on what animal model of thrombosis you look at, you can come up with different conclusions as to whether the tissue factor is coming from the vessel wall or the blood. So I think that you may be looking locally at the plumbing, and we may be looking at the water quality. It may be a water quality issue here. It may be

a late deposition of tissue factor from circulating microparticles. I think that bears looking at.

Boxer, Ann Arbor: What is the mechanism of microparticle generation and particularly in inflammation as related to cytokine release, and can you inhibit it with cytokine inhibitors?

Key: That's a good question. There is no doubt that directly treating monocytes, either in whole blood or isolated with LPS and other agents, will initiate a series of events that will lead to microparticle production. The common denominator seems to be an increase in intracellular calcium and degradation of the cytoskeleton and release of microparticles following the activation of scramblase, which is calcium-dependent, and inhibiton of the aminophospholipid translocase system. So, in inflammatory cells, yes you can induce it with cytokines, and you can block it with some of the Th2 cytokines; for example, IL-4, IL-10 are actually fairly potent inhibitors of that process.

Willerson, Houston: For the cardiologists: you can overexpress TFPI with a viral vector locally at sites of vascular injury in atherosclerotic and non-atherosclerotic arteries, and it is very protective against both thrombosis and restenosis—particularly if you overexpress it concomitantly by simultaneously overexpressing prostacyclin. It's a very potent combination in preventing thrombosis and we are trying to introduce that form of gene therapy in humans now.

Key: Thank you for the comment. I think that helps to essentially get at the question earlier of where to direct the action—to the vessel wall or to circulating tissue factor.