[Frontiers in Bioscience E4, 289-299, January 1, 2012]

# Tissue factor in blood cells and endothelial cells

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## 1. ABSTRACT

There are contradictory reports about the synthesis and expression of TF antigen/activity in blood cells and plasma and in the surrounding environment of endothelial cells. In this review article we focus on the many divergent findings on the expression and presence of TF in various blood cells, plasma and endothelial cells in normal and pathological states. The widespread use of antibodies with inferior and misleading specificity and TF activity assays with low sensitivity/specificity, may be the major explanation for all the controversy. Based on our own experience and many other recent reports, pertaining to the concept of so called blood borne TF, we conclude that TF is exclusively expressed in and associated with circulating monocytes in blood of healthy individuals. However, several pathological conditions are associated with activated monocytes, shedding TF-rich microparticles that are transferred to activated platelets, neutrophils, and endothelial cells.

## 2. INTRODUCTION

The distribution of tissue factor (TF) in blood vessels has been investigated immunohistochemically, and it is suggested to be localized predominantly in cells of the adventitia and thus shielded from the vessel lumen. This presence of TF amounts to a hemostatic envelope encapsulating the vascular bed, the disruption of the integrity of which would instantly trigger the clotting process (1, 2).

Two major discoveries led to the emergence of the notion of blood borne TF. Initially it was observed that when blood of healthy individuals is allowed to pass along a collagen-coated glass slide *ex vivo*, a TF-dependent thrombus is formed (3), and this was followed up by a study demonstrating that monocytes and possibly PMN leukocytes were involved in the transfer of TF particles to platelets (4).

In a third, remarkable study supporting the concept of blood borne TF, it was shown that laser-induced injury to the vessel wall in a mice model led to instant platelet adherence and activation, followed by a very rapid appearance of TF associated with the platelets (5,6). Further, it was demonstrated that this TF deposition occurred more rapidly than the binding of white cells to the platelet/thrombus (7). Since a previous study from the same authors had shown that the TF active in forming the thrombus was derived from the hematopoietic system and not the vessel wall (6), it was suggested that the TF triggering the thrombus formation, had to be present in plasma and probably associated with circulating microparticles (7). In contrast to this observation in the microvascular system, the site of TF exposure and triggering of thrombus formation upon injuries to the macrovascular system, was revealed to be the cells present in the vessel wall (8).

The objective of the present review is to give an update of the localization of TF in the blood organ in physiology and pathophysioloy, and thereby also provide a plausible explanation for the reports on which the concept of blood borne TF is based.

## 3. THE PRESENCE OF TF IN MONOCYTES

The first documentation that monocytes may synthesize TF was reported by Rievers *et al* in 1975 (10). Here it was shown that endotoxin induced TF activity in isolated monocytes, whereas only traces of activity were detected in granulocytes and lymphocytes, probably deriving from contaminating monocytes as suggested by the authors.

TF has also been reported to be present in resting monocyte cell lines (11). More recently we found that 1-2 % of the CD14 positive cells of monocytes, freshly isolated from the blood of healthy individuals, possessed inactive TF (12), subject to activation upon stimulation with a calcium ionophore (13). This kind of phenomenon has been referred to as an encryption-decryption transition of TF. Encryption of TF has been suggested to account for the observation that although most of resting monocyte TF is (already) available on the extracellular surface, only 10 – 15% of it has biological activity. The presence of phosphatidylserine (PS) is required for the activation of FIX and FX by the FVIIa-TF complex, and clusters of PS may be needed for exertion of the proteolytic cleavage of the substrate proteins (14). The calcium ionophore effect is mediated through a reorganization of the phospholipids in the cell membrane, whereby PS becomes exposed on the outer surface and thus available for rendering the TF-FVIIa complex active. The demand for very high local PS concentrations may probably be the explanation for the so called encryption/decryption phenomenon. In another suggested mechanism for the conversion of inactive TF in the cell membrane to an active form, a role has been posited for PDI through its conversion of unpaired cysteine thiols into the formation of the disulfide bond Cys186 –Cys 209(15). Recently it was claimed that the procoagulant activity of soluble and microparticle-associated TF is enhanced by bovine liver -derived PDI (bPDI), independently of PDI's reductase and isomerase function (16). In still another study from the same group it was suggested that the effects of bPDI are distinct from the rate-enhancing effect of phospholipids (17). However, the involvement of PDI as part of making cell-associated TF active is controversial and has been questioned in studies demonstrating lack of a PDI role (18).

Monocytes in whole blood may be stimulated by LPS, activated complement or activated complement fraction C3b and C5a (19, 20). Many of the other products known to induce TF in monocytic cell lines, are only capable of enhancing the generation of TF in already activated monocytes of whole blood (for review see 21).

The expression of TF in monocytes of whole blood after stimulation varies tremendously between individuals (22), and appears to be an inherited trait that at least partially is associated with high production of cytokines (23). The very high expression of TF seen in some test samples, is at least partially dependent on the interaction between monocytes and platelets (24). The platelet effect on TF expression in monocytes is granulocyte dependent in a P-selectin (on activated platelets) and PSGL-1 (on monocytes) mediated reaction (24). The major regulators of LPS-induced TF activity in monocytes of whole blood were found to be the platelet activating factor (PAF) receptor, the thromboxane $A_2$ receptor and a proteinase (inhibitable by Pefablock) ) (25). At the level of gene regulation, NFkB and AP-1 play central roles (26).

In-cell Western assay of TF expression in resting and stimulated monocytes of whole blood revealed inter-individual differences in the localization of TF in the cells (12). So-called high responders (expressing very high levels of TF in stimulated monocytes) possessed a higher percentage of TF on the surface of the cells than did low responders, which may play a role in TF-induced thrombosis, e.g. post-operatively, when TF expression is enhanced (27, 28). The TF located intracellular is probably inactive until it merges with phospholipids in the cell membrane.

Many studies have shown that TF expression is enhanced in various diseases associated with activated monocytes (for review see 29). This is particularly evident in cases where strong agonists are present in the blood, e.g. during meningococcal infection, where it has been shown that high level of TF present in circulating monocytes upon patients' presentation at the hospital, was predictive of death (30). Low grade TF expression has been reported in cardiovascular disease, diabetes, Lupus anticoagulant, cancer and many other diseases where monocyte activation is part of the pathophysiology of the disease (31). Recent reports have indicated that TF present in blood cells may also play a role in venous thromboembolism (VTE) (32).

## 4. TF IN NEUTROPHILS

Niemetz and Marcus reported in 1974 on a stimulatory effect that platelets and platelet membranes had on the procoagulant activity of rabbit leukocytes, but no differentiation between leukocyte types was made (33). A study from Nemerson`s laboratory, after they had launched the notion of blood borne TF, suggested that in their *in vivo* model TF might be associated with neutrophils, since it was shown to be transferred from monocytes and possibly neutrophils to platelets in a CD-15 dependent reaction (3). Although neutrophils harboring TF were repeatedly observed, the authors concluded that they did not as yet know whether this TF was endogenously synthesized by the neutrophils or represented engulfed exogenous material subsequently transported to the site of thrombus growth. In a follow-up study from this group it was found that thrombogenic TF on leukocyte derived microparticles became incorporated into spontaneously forming human thrombi (4). It was suggested that monocytes and possibly polymorphonuclear (PMN) leukocytes are the sources of circulating plasma TF, with the potential of being transferred to platelets, in effect producing TF-positive platelets capable of triggering and propagating thrombosis. This transfer process was shown to be mediated by the interaction of CD15 with platelets, and also by TF, which seems to act as an adhesion molecule (3,34). This observation accords with the findings that anti-CD15 antibodies abolish about 80% of LPS-induced TF activity in monocytes of cell suspensions recombined with platelet-rich plasma (34).

The question whether human neutrophils by themselves are capable of synthesizing and expressing TF was addressed in another study using whole blood system as well as isolated blood cell fractions. Isolated granulocytes recombined with heparinized plasma failed to express any significant amounts of TF antigen or activity when stimulated with LPS, LPS in combination with phorbol myristate acetate (PMA), or tumor necrosis factor-alfa (TNF-alfa) (35). However, when heparinized whole blood was subjected to LPS+PMA stimulation for 24 hours, the isolated granulocyte fraction contained some TF activity as well as antigen. The small amount of TF activity and antigen associated with the granulocytes was most probably due to monocyte-dervied TF-rich microparticles, which strongly bind to granulocytes (36).

In contrast to the findings of the latter study, there are several reports on the presence of TF in neutrophils in animals undergoing sepsis (37). In agreement with these reports, Maugeri *et al* (38) claimed that isolated human granulocytes produce and express functional TF upon stimulation. Although expression of TF antigen was detected by flow cytometry 3 min after the cells were stimulated with P-selectin or formyl-MetLeuPhe (fMLP) and TF activity after 2 min incubation with these stimuli, the authors argued that TF is not constitutively present in peripheral granulocytes, but only produced upon stimulation and specific gene transcription. They hypothesized the unlikelihood that TF acquired from platelet- or monocyte-derived microparticles should be the source of TF in the granulocytes, since these cells are remarkably phagocytic. One may therefore question the reliability of these observations, on the ground that detectable amounts of newly synthesized protein are very unlikely to appear within a time frame of 2-3 min, as seen in this study.

In contrast to the study above, Egorina *et al* (39) failed to detect TF expression in granulocytes, in agreement with the previous report from Osterud`s laboratory (35). Interestingly, granulocytes isolated from stimulated whole blood contained small amounts of TF activity, whereas plated resting or LPS/PMA stimulated granulocytes did not possess any TF activity (39). This suggests that granulocytes may acquire TF, but do not synthesize it by themselves. In experiments where whole blood was reconstituted with TF-silenced monocytes and then stimulated with LPS, the minute TF activity normally associated with granulocytes was even further reduced, to nearly non-detectable levels. The acquisition of monocyte-expressed TF-yellow fluorescent protein (TF-YFP) fusion protein by granulocytes in whole blood, further corroborated the validity of the hypothesis of transfer of TF from monocytes to granulocytes in the blood. Furthermore, from the results using the membrane-targeted myr-YFP, which was not transferred from monocytes to granulocytes under conditions of LPS-stimulated whole blood, it was concluded that the TF-YFP transfer is specific to TF and not a phenomenon arising randomly from membrane fusion processes involving shedded monocyte material in concert with other cells (39).

In line with the above observations, an *in vivo* study by de Waard *et al* (40) based on a mice model, showed that the clusters of TF-protein positive cells detected in the spleen were predominantly granulocytes, but no TF mRNA expression was observed in these cells. Based on these observations and the presence of TF-protein positive granulocytes detected after splenectomy, it was hypothesized that granulocytes take up TF for transport to other locations, in order to initiate fibrin formation or induce pro-inflammatory gene expression upon interaction with factor VIIa.

It is well documented that activated complement factors, notably C5a is a potent inducers of TF in monocytes (20, 41). Further, Ritis *et al.* (42) demonstrated that antiphospholipid (aPL) antibody, activating complement downstream signalling via C5a receptors, induced TF expression in isolated neutrophils incubated with human serum. Since it is quite difficult to obtain isolated neutrophils that do not to some extent remain contaminated by monocytes, conceivably this might explain the increase in TF mRNA observed by these authors. Further, in their system monocyte-derived microparticles carrying TF induced by C5a in the

monocytes may have been transferred to the neutrophils, accounting for the neutrophil-associated TF observed in their FACS measurements. Almost simultaneously Redecha *et al* (43) reported that in response to aPL-provoked C5a in mice, isolated neutrophils from these mice expressed TF. However, one cannot exclude a scenario of monocyte origin for this TF, involving monocyte derived TF-positive microparticles binding to activated neutrophils in the circulation. We failed to see any induction of TF in neutrophils when isolated cells were incubated with IgG from patients with lupus anticoagulant, in contrast to the very high level of TF induced by the same IgG in monocytes of whole blood (44).

## 5. TF IN EOSINOPHILS?

Even though granulocytes in circulating blood appeared to be free of TF and also failed to synthesize TF when stimulated, Moosbauer *et al* (45) reported on expression of TF antigen as well as activity in isolated and stimulated eosinophils. Mature eosinophils were found to express considerable amounts of TF, which in resting eosinophils was located preferentially in the specific granules. Platelet-activating factor (PAF), and more effectively so granulocyte-macrophage colony-stimulating factor (GM-CSF) plus PAF, provoked translocation of preformed TF to the eosinophil cell membrane. The combination GM-CSF/PAF was also shown to increase the TF transcript levels. The activated eosinophils exhibited TF procoagulant activity amounting to up to one third of that of LPS-stimulated monocytes.

In contrast to this report, Sovershaev *et al* (46) found by FACS analysis of resting and stimulated eosinophils no revelation of TF antigen expression on the cell surface. Furthermore, immunoblotting and testing for procoagulant activity of eosinophil lysates did not show any TF protein or TF activity under resting or stimulated conditions. In contrast, monocytes stimulated in plasma or medium, possessed significant levels of TF antigen on the cell surface as well as released in cell lysates, as readily detected by FACS and immunoblotting. This TF antigen expressed activity in a TF activity assay. In resting or stimulated eosinophils, no TF mRNA level of any significance could be detected using real-time polymerase chain reaction (PCR), whereas in monocytes TF mRNA levels were significantly increased after stimulation.

## 6. MICROPARTICLES

For several years it has been well known that TF-rich microparticles are part of the pathophysiology of several diseases (47). In addition, TF antigen has been measured in plasma of healthy subjects as well as patients with various diseases. Unfortunately, test systems for TF antigen have turned out not be of reliable specificity, implying that in most studies on TF antigen in plasma, the reported concentrations are probably manyfold higher than the true values.

Two forms of TF have been proposed to exist in plasma, a soluble, truncated form, lacking the membrane-spanning and intracellular sequence, and full length TF antigen associated with microparticles of blood in healthy subjects. In accordance with this notion, we reported substantial expression of TF in microparticles (36), but realized later that the secondary antibody that we had used reacted non-specifically with the human heavy chain of IgG present in the test sample. In a follow up study, no TF activity or antigen (ELISA) were detected in isolated microparticles from blood of healthy subjects (48).

The currently emerging notion that microparticles in healthy blood are lacking TF, which accords with the inability of TF antibody to inhibit the clotting of plasma/blood, does not fit in well with the interpretation offered in studies on mice subjected to laser induced injury, that circulating TF is the likely trigger of thrombus formation (6,7). In this particular model it was found that TF antigen appeared early on activated platelets present at the injury site (peaking at 100 sec) and before white cells became attached to the thrombus. It was indicated that TF derived from hematopoietic cells is delivered in microparticle bound form during the initial phase of thrombus development (7) . However, unless mice differ from humans by possessing TF rich microparticles, the TF available for local deposition must rather be a soluble form, which is only active if FVIIa is also present (49). Absent of FVIIa, any detection of TF on the thrombus is likely to be non-specific. The same considerations are of relevance for the mechanism whereby thrombus is formed when freshly drawn human blood is allowed to pass along the surface of collagen-coated slips as described earlier. Is there an active soluble TF which can not be detected in whole blood or is the TF released from the puncture of the veins?

When monocytes are activated and express TF, the shedded microparticles are TF-rich and when transferred to platelets are making these extremely hyper-thrombotic (34, 50). Such interaction between microparticles and the platelets is dependent on PSGL-1 on the MPs and P-selectin exposed on activated platelets, and appears to involve a fusion between the TF-rich microparticles and the platelets (50). Thus, in any type of disease where monocytes are activated, circulating free MPs or MPs associated with activated platelets express TF

## 7. TF IN PLATELETS?

Much like the issue whether granulocytes express TF, the question whether platelets have such a capacity is steeped in controversy, even to a greater extent. Already in 1974 platelets were suggested to play a role in the regulation of TF activity by leukocytes after stimulation with endotoxin (LPS) (33). The first report on TF presence and expression in platelets was by Zillman *et al* (51), where they detected TF antigen and activity on platelets adhering to leukocytes after 5 min

stimulation of whole blood with collagen. An extension of this study revealed TF stored within alfa-granula and the open canalicular system of the platelets (52). Following activation with either collagen or thrombin, TF activity was exposed on the platelet membrane. Other reports have corroborated the concept of the presence of TF in platelets. Thus, Siddiqui *et al* (53) found that collagen-stimulated platelets exposed TF activity, although the specificities of their assays are in question. In line with this report, Camera *et al* (54) claimed the presence of functionally active, membrane-associated and immune-reactive TF in activated platelets of healthy individuals and detectable TF mRNA in un-stimulated platelets. These studies obviously do not take into account any role played by de novo protein synthesis.

An interesting observation was made by Schwertz *et al* (55), who found that quiescent human platelets express TF pre-mRNA in response to activation. This precursor was spliced into a mature TF mRNA, the process being associated with increased TF protein expression, pro-coagulant activity, and accelerated thrombin generation. In agreement with this report on the synthesis of TF in platelets, Panes *et al* (56) claimed that TF was present in quiescent platelets and that it was enhanced by their activation by TRAP. Surprisingly, neo-synthesis of TF by platelets was reported not only in activated platelets but also in resting platelets. More recently, the same research group proposed that only von Willebrand factor (vWF) together with ristocetin might induce TF activity in platelets in a short time and that cell membranes needed to be intact (not lysed) in order for TF activity to be detectable (57).

The above results have not resolved the persisting controversy of the presence, synthesis and functional activity of TF in platelets. In contrast to the reports above, we have failed to detect TF antigen or activity in resting platelets or platelets of whole blood stimulated with LPS, LPS in combination with PMA (48) or lupus anticoagulant antibodies (IgG), the latter being more effective in inducing TF expression in monocytes than is LPS (44). Similarly, TRAP activation of whole blood or isolated platelets in the absence or presence of LPS, failed to induce any TF antigen that could be detected in an ELISA assay or by its activity. In the same way, isolated platelets stimulated with collagen or TRAP failed to induce detectable TF activity and antigen (Østerud and Olsen, unpublished data).

Recently, Bouchard *et al* (58) reported further evidence of the absence of TF expression in platelets, on the basis of flow cytometric analysis of PAR1 and PAR4 activated platelets. Despite maximal alfa-granula release, no TF antigen was detected on the platelets, in accordance with their earlier observation on A23187-induced platelet activation. Supporting the lack of TF expression in platelets was recently demonstrated by the failure to detect expression of pre-mRNA or mRNA in mouse platelets (59)

## 8. TF EXPRESSION IN ENDOTHELIAL CELLS?

Although association of TF with plasma membranes of vessel wall was reported by Zeldis *et al* in 1972 (60), the antibody that they had used turned out highly poly-specific and these early conclusions were eventually withdrawn in 1978. On the other hand, TF coagulant activity of cultured endothelial cells, as well as SMCs, was reported in 1977 (61). Observations of TF in cultured human endothelial cells (HUVECs) were corroborated by several groups (for review see 62), and it was also demonstrated that thrombin was an agonist of TF induction in HUVECs (63). Later it was reported that IL-1-beta and TNF-alfa might induce TF in ECs. (64). It should be pointed out that to make ECs available for *in vitro* studies, exposure to relatively harsh treatment such as collagenase digestion is required, in a process normally taking four days. The cells adapt by developing traits that deviate significantly from those of the original resident ECs, the facility of| TF expression apparently being one such trait.

In the early eighties we experienced a high incidence of severe meningococcemia in Northern Norway (65). The fulminant DIC seen in the patients appeared to be associated with massive cellular activation, as confirmed by induced TF activity in circulating monocytes and major impair of blood flow (66). We concluded at that time that if endothelial cells *in vivo* could be stimulated to express TF at the same level as found in LPS-stimulated ECs *in vitro*, any patient exposed to gram negative bacteria would have been in a precarious situation, unless some super-efficient inhibitory system was also present. This led us to further investigations, using a rabbit endotoxemia model entailing subjection of the animals to Schwartzman reaction by the intravenous injection of two doses of LPS at 24 hrs interval, a regimen that failed to provoke any detectable TF expression in the ECs (67). A similar finding was reported by Erlich *et al.* (68) in a rabbit given intravenous injection of endotoxin. In contrast, Semeraro *et al* (69) found enhanced TF expression on the endothelium of rabbits given endotoxin. In retrospect, however, their finding might be explained by the presence of TF-rich microparticles bound to the endothelium.

The failure to see any expression of TF on the endothelium of severely infected rabbits, prompted us to explore isolated segments of human vessels. Thus, saphenous veins collected from coronary artery bypass patients were perfused with endotoxin (30ng/ml) or thrombin (2.5 U/ml) for 3 hrs at 37 $^{o}$C (70). Subsequently the veins were treated with collagenase, and the ECs collected and subjected to TF activity measurements using our highly sensitive and specific TF activity assay (71). Some intact, perfused veins were examined for the presence of any TF activity on the vessel wall, by allowing FVII/FVIIa and FX to interact at the lumen surface of the vessel, followed by the quantification of FXa generated in a chromogenic assay. No TF activity was detected, whether in ECs detached by collagenase treatment or at the endothelial surface of vessel segments that had only been subjected to perfusion treatment.

Evidence in favour of the notion that TF may be expressed in endothelium *in vivo* was found by Contrino *et al* (72). Using monoclonal antibodies and a novel probe for functional TF, they detected TF localized to the vascular endothelial cells (and tumor cells) within the tumors of seven patients with invasive breast cancer. One might argue that since it is now well established that cancer tumors are generating significant amounts of TF-rich microparticles, the TF detected on the endothelium might conceivably be located in monocyte/macrophage derived TF-rich microparticles, bound to the activated endothelium through the interaction of PSGL-1 on the microparticles with P-selectin exposed on the endothelial surface.

Recent reports of TF in the endothelium of sickle cell anemia patients, along with the detection of blood borne TF-rich microparticles of EC as well as monocyte origin, have rekindled the notion that ECs may synthesize TF (73,74). However, in a study of TF expression in sickle cell transgenic mice, Solovey *et al* (75) found using immunostaining, that endothelial TF expression was confined almost exclusively to the pulmonary veins. Since the sickle cell trait is associated with monocyte activation, a conceivable scenario is that monocyte derived TF-rich microparticles bind to and fuse with activated ECs as well as with EC derived microparticles exposing P-selectin, that may interact with PSGL-1 on the monocyte derived microparticles. In accordance with this, Del Conde *et al* (50), showed that TF-rich microparticles from monocytes not only bound to activated platelets, but also fused with them, in effect transferring both surface proteins and lipids to the platelet membrane.

Further support for such a concept was presented by Drake *et al* (76), in an immunohistochemical investigation of TF expression in a baboon model involving lethal E. coli sepsis. Expression of TF by ECs became detectable only in the splenic microvasculature, where endothelial specificity of TF expression was confirmed by dual immunofluorescence of TF together with van Willebrand's factor (vWF) and with thrombomodulin (TM) . Although the TF detected was associated with the ECs, this does not exclude the possibility that it ultimately may have been derived from activated circulating monocytes expressing TF. Thus it is well established that in severe sepsis, TF-rich microparticles of monocyte origin are circulating in substantial quantities and these may be bound to activated endothelium exposing P-selectin on the surface.

The observations using the baboon model related above were further re-examined and extended (77). In a similar baboon model of E. coli sepsis it was shown that TF was localized preferentially to vessel branches, EC surface, leukocytes, and platelet aggregates and accumulated in large amounts in the sub-endothelial space. It was shown that branched segments of aortas contained higher TF protein levels and coagulant activity than did equivalent linear regions. TF was found to be located on the endothelial surface (distributed in a granular pattern), on platelet-rich micro-thrombi and on adherent or transmigrated leukocytes. It was concluded that it was not possible to determine whether TF was synthesized locally or acquired from circulating particles via P-selectin/PSGL-1 interactions. Since part of the particles also contained PSGL-1, this was suggested as a strong indication that leukocyte-derived microparticles might deliver TF to the EC surface.

Murine models have been very important in search for biological roles of TF *in vivo*. In such models, TF has been found to be mandatory for life in that it prevents lethal bleedings and facilitates development of new arteries (angiogenesis). In a recent study TAT levels were measured in endotoxemic mice that overexpressed a mutant I-kBa, an inhibitor of NFkB, selectively on the endothelium (78). The endothelial NF-kB blockade inhibited TF expression in ECs but did not inhibit LPS-induced TF expression in heart, kidney and liver. Since the NF-kB blockade in the ECs is associated with a reduction in inflammation which attenuates the activation of the coagulation in animals of sepsis (79), the reduction in TAT might not have been a result of reduced TF expression by ECs as suggested by Pawlinski *et al* (59). In laser induced injury models of the microvasculature, the hematopoietic cells generating TF have been shown to be essential for the development of spontaneous thrombi (6), whereas upon injury to the macrovasulature system, induced thrombi were shown to depend on TF present in the vessel wall (8). In a further development using organ specific knock-out of TF, it was recently claimed that thrombus formation provoked by laser induced injury to the microvasculature depended on hematopoietic and endothelial cells (80). This partially contradicts the findings of another excellent report, by Pawlinski *et al* (59), who showed that high dose LPS (5 mg / kg body weight) was associated with high generation of thrombin–anti thrombin complex (TAT). By using mice with cell-type specific gene deletion (TFf/f, LysMCre and TFf/f, Tie-2Cre mice) and mice expressing human TF (HTF mice to avoid embryonic death), it was shown that low expression of TF in the hematopoietic or non-hematopoitic cells, reduced the inducible plasma TAT levels by 47% and 39%, respectively. However, when only the ECs had reduced TF expression, no reduction of TAT levels was observed. It was concluded as implications of their findings that in this model both the hematopoietic and non-hematopoietic cells contributed to the activation of coagulation during endotoxemia, and that the deletion of the TF gene in myeloid cells but not in ECs significantly reduced the activation of the coagulation. The role of the

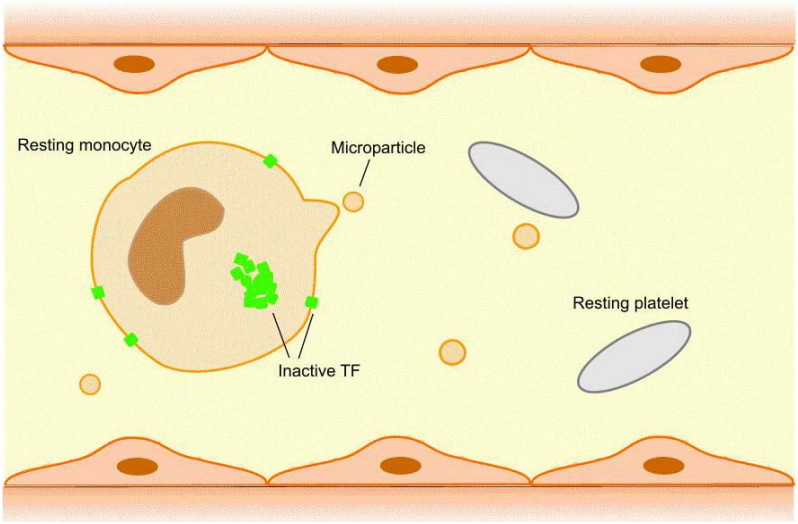


**Figure 1.** TF is exclusively present in monocytes of circulating blood cells of healthy individuals. About 1-2% of CD14 positive cells contain TF which mainly is localized in clusters in the interior of the cell and only a small portion is found in an inactive form in the cell membrane. Treatment of these resting monocytes with Ca-ionophore causes a transfer of the TF to the surface in an active form.

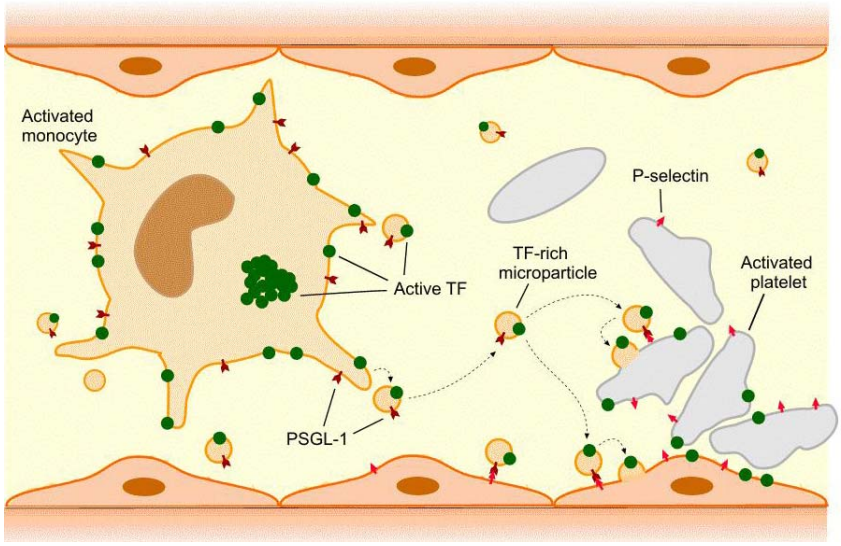


**Figure 2.** Activated blood *in vivo*. Monocytes express large amounts of TF present intracellularly as well as exposed on the surface of the cell membrane. TF-rich microparticles derived from monocytes are formed which binds to and fuses with activated platelets and endothelial cells in a PSGL-1 –P-selectin dependent reaction. PSGL-1 is located on the microparticles whereas P-selectin becomes exposed on activated platelets and endothelial cells. Neither platelets nor endothelial cells synthesize TF, but acquire TF from monocytes. Neutrophils also bind TF-rich microparticles but never express TF themselves (not shown)

hematopoietic cells, fibroblasts, SMCs, pericytes etc. in expressing TF, most likely hinges on enhanced availability due to induced membrane permeability changes and leakage between the endothelial cells, changes known to be directly caused *in vivo* by relatively high concentrations of LPS.

## 9. SUMMARY

In summary, our current view of concept of the so called blood borne TF, is that TF is exclusively expressed and associated with monocytes in healthy individuals. The TF activity in the relatively few monocytes expressing TF is protected from exposure to blood through the lack of activity unless the monocytes become activated. However, several pathophysiological conditions are associated with activated monocytes whereby TF-rich microparticles are formed that may be transferred to activated platelets, neutrophils and endothelial cells. These latter cell-types are probably never synthesizing TF themselves, but may aquire TF from monocyte derived TF-rich microparticles (Figures 1 and 2).

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**Key Words:** Blood cells, Endothelial cells, Monocytes, Neutrophils, Platelets, Tissue factor, Review

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