The role of monocytes in thrombotic disorders
Insights from tissue factor, monocyte-platelet aggregates and novel mechanisms

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Summary
Although, the main physiological role of monocytes is attributed to innate immunity (that is, phagocytosis) and the development of tissue macrophages and dendritic cells, the pathophysiological role of these goes far behind these (simplistic) limits. Indeed, monocytes constitute a major source of blood tissue factor, a key element of the extrinsic coagulation cascade. Monocytes actively bind to platelets, thus forming very prothrombotic mono-

cyte-platelet aggregates. Additionally, these cells link inflammation and the procoagulant state observed in various pro-thrombotic conditions. However, monocytes are also crucial for successful thrombus recanalisation. In this article, we review the available data on potential mechanisms that link monocytes with thrombosis-related processes.

Keywords
Monocytes, thrombosis, tissue factor, monocyte-platelet aggregates

Introduction
Thrombosis-related disorders represent a major challenge in current medical practice. The annual incidence of hospital admissions for acute coronary syndromes (ACS) is well over three per 1,000 inhabitants in developed countries and has been associated with a high mortality and progression to heart failure (1, 2). Venous thromboembolism affects more than one million people in Western Europe and approximately 900,000 individuals in the US annually with 670,000 fatalities each year in the US and Western Europe caused by pulmonary embolism (3, 4). Unfavourable outcomes following thrombotic conditions are common despite the current availability of a range of anticoagulant and antiplatelet drugs.

Unfortunately, the development of novel, more potent antithrombotic therapies directed to inhibit activity of platelets or coagulation factors are limited by the correspondently increasing risk of bleeding complications (5). This motivates the search for new alternative targets for future antithrombotic treatments. What might they be? Classically, two major elements of the coagulation system are represented by various coagulation factors and platelets. However, accumulating evidence supports the important role of other blood cells, such as monocytes, in the thrombosis-related processes.

The main physiological role of monocytes is attributed to innate immunity (that is, phagocytosis) and the development of tissue macrophages and dendritic cells, but the pathophysiological role of these goes far behind these (simplistic) limits. In this article, we review the available data on potential mechanisms that link monocytes with thrombosis-related processes.

Monocytes and tissue factor activity
Tissue factor (TF) is a key element of the extrinsic coagulation cascade. TF exists in two forms: (i) as a transmembrane protein, which can be activated during vascular wall damage and exposure of subendothelial tissues to the blood circulating elements, and (ii) as a splice variant in soluble form. In addition to its well-established role in coagulation, TF participates in other cellular processes (6). The latter includes vascular wall and plaque remodelling by the coordination of migration and proliferation of vascular smooth muscle cells (7). Furthermore, TF may play a role in the process of angiogenesis, given that it is crucial for the development of embryonic blood vessels in mice and...
amplifies tumour neovascularisation (6, 8). Importantly, blood itself contains substantial amounts of TF (e.g. in soluble form) and is able to trigger coagulation, even with endothelial integrity virtually preserved.

Importantly, monocytes appear to be the major source of blood TF. Monocytes expose considerable quantities of TF on their surface being stimulated by a variety of inducers of which lipopolysaccharides (LPS) and C-reactive protein (CRP) are especially important (Table 1) (9, 10). Admittedly, the ability of these inducers to stimulate monocyte TF expression was largely tested ex vivo with related methodological biases (e.g. a disputable purity of commercially sourced CRP), and thus, may not accurately reflect their (patho)physiological consequences in vivo (11–13). The formation of TF in monocytes requires transcriptional activation of the corresponding gene with two different TF mRNA species being detected (14, 15). The amount of TF mRNA preformed by resting monocytes does not significantly affect the surface density of this protein but nonetheless, this appears to be responsible for the substantial variation of surface TF expression following stimulation by LPS (16).

Monocyte transmigration through the arterial wall to tissues prior differentiation into macrophages (e.g. adhesion to endothelial cells or smooth muscle cells) is also associated with enhanced TF generation (17). Moreover, monocytes stimulate TF

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APS, antiphospholipid syndrome; IL, interleukin; LPS, lipopolysaccharides; LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for binding herpesvirus entry mediator on T cells.
expression by endothelial cells, thus contributing to the supply of the tissue with this key factor of extrinsic pathway of coagulation. Additionally, given that megakaryocytes do not express TF but the factor is present on platelets in circulation it is possible that TF-bearing monocyte-derived microparticles may be involved in transfer of TF to platelets in addition to the production of the factor from stored intra-platelet mRNA (18, 19). Also some controversy still exists, monocyte appear to produce little TF pathway inhibitor at rest or following stimulation with LPS (10).

**Monocyte TF activity and cardiovascular risk factors**

The number of studies have demonstrated a close association between the TF-forming activity of monocytes and various cardiovascular risk factors (Table 2). Indeed, more TF is produced by monocytes in subjects with hypercholesterolemia while this activity might be reverted by administration of statins (20). In patients with diabetes mellitus, an increased generation of TF by monocytes closely corresponds to levels of advanced glycosylation end products (21). Serum amyloid A, a ligand for the receptor for advanced glycation end products (RAGE) has also been found to be a potent and rapid inducer of human monocyte TF (22). Hyperglycaemia-hyperinsulinaemia induced monocyte TF expression in healthy individuals (23). In contrast, insulin inhibits TF expression in monocytes and monocyte-derived microparticles in patients with diabetes (24).

Angiotensin II, a major pathogenic factor of arterial hypertension, stimulates TF expression in monocytes through the angiotensin II type I receptor (25, 26). Consistently, angiotensin converting enzyme inhibitors suppress endotoxin-induced TF expression in monocytes (at least in vitro) and reduces TF plasma activity in hypertensive patients (27–29). Smoking has been shown to increase formation of TF by monocytes in pre-menopausal women, especially in those using oral contraceptives (30). The induction of TF expression by circulating monocytes has been suggested as a potential mechanism by which homocysteine may induce thrombosis (31).

**Monocyte TF activity and coronary artery disease**

Multiple factors seen in coronary artery disease (CAD) promote excessive monocyte TF formation the accords with the prothrombotic state typical for the disease. Indeed, extracts of human atherosclerotic plaque significantly enhance monocyte TF expression (32–34). Also, a ligand in the tumour necrosis factor superfamily called LIGHT (lymphotoxinlike inducible protein that competes with glycoprotein D for binding herpesvirus entry mediator on T cells) is known to be increased in patients with CAD – especially in those with unstable angina – and is a potent mediator of TF expression in human monocyte-derived macrophages (35). In fact, excessive monocyte TF production may partly explain the high levels of circulating TF seen in patients with CAD. For example, TF formation by monocytes from healthy donors has been shown to be enhanced by incubation in the presence of plasma from patients with CAD (36).

The increased expression of TF by monocytes following stimulation by CD40 is accompanied by up-regulation of gener-

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Table 2: Monocyte tissue factor expression and monocyte-platelet aggregates in patients with cardiovascular risk factors and cardiovascular disorders.
tion matrix metalloproteinases (MMPs), a recognised promoter of atherosclerotic plaque instability (37). These findings may justify potential association between high monocyte TF expression and acute coronary syndrome (ACS) development. However, the currently available data on monocyte TF-expression in ACS are controversial and further studies are required (38, 39).

In vitro, the presence of a stent causes a significant increase in TF expression in monocytes attached to platelets. Indeed, monocyte are the main source of TF depositing in the in-stent thrombus in this model thus suggesting possible link between monocyte activity and risk of stent thrombosis (40). Of interest, monocyte TF expression can be inhibited by the macrolid rapamycin, which is commonly used for drug-eluting stents for restenosis prevention (41).

**Monocyte TF activity and venous thrombosis**
The available data indicate the role of monocyte-derived TF in the development of the venous thrombosis. Levels of monocye-bound TF are significantly elevated in patients with deep venous thrombosis (DVT) and are sensitive and specific markers for DVT detection (42). Increased monocyte TF activity has also been reported in patients with cerebral venous thrombosis (43). Importantly, TF generation and release seems to be the principle mediator of monocyte impact on venous thrombus generation as direct monocyte procoagulant activity nor the number of D-dimer-positive monocytes was found to correlate with the occurrence of DVT (44).

Monocytes are also associated with other prothrombotic conditions. In patients with heparin-induced thrombocytopenia, antibodies to heparin-platelet factor 4 complex induce TF synthesis by monocytes and increase monocyte procoagulant activity (45). Additionally, the increased capacity of monocytes to generate TF in patients with essential thrombocythaemia and polycythaemia vera is associated with the activation of the coagulation system and subsequent risk of thrombotic complications (46, 47).

**Monocyte TF activity in antiphospholipid syndrome and other prothrombotic conditions**
Antiphospholipid syndrome is characterised by vascular thrombosis in the presence of antiphospholipid antibodies. A number of studies have shown associations between antiphospholipid syndrome and activation of circulating blood monocytes and increase their procoagulant activity, inducing the expression of procoagulant activity of TF (48–50). The cell-surface expression of TF on monocytes is further increased in the patients with primary antiphospholipid syndrome with thrombosis compared to those free from thrombotic complications (51–53). In-vitro studies have also shown that plasma from patients with antiphospholipid antibodies and a history of thrombosis is much more potent stimulator of monocyte TF expression compared to plasma from patients with antiphospholipid antibodies without thrombotic events in anamnesis (54). The ability of beta2-glycoprotein I to stimulate monocyte TF expression in patients with antiphospholipid antibodies was even suggested as a potentially useful predictor for antiphospholipid syndrome (55). At a molecular level, antiphospholipid antibodies may interact with specific cell surface receptors (e.g. FcγRII or annexin II), with consequent induction of downstream regulating signals, and that ultimately results in up-regulation of monocyte TF expression (56, 57).

**Monocyte-platelet aggregates**
The adhesion of leukocytes to platelets deposited at the site of vascular injury may represent an important mechanism by which leukocytes contribute to haemostasis and thrombosis (58). Indeed, the risk of graft occlusion after reconstructive surgery in patients with peripheral vascular disease is much higher in those with more active leukocyte-platelet adhesion in the early postoperative period (59). The number of monocyte-platelet aggregates (MPA) is also markedly increased after major joint arthroplasty (60). Such an increased formation of MPA has been associated with high levels of P-selectin, CD40 ligand, TF and Mac-1 expression on monocytes; a correlation with beta-thromboglobulin levels suggests a role of monocyte-platelet conjugates in augmenting blood coagulability after major joint surgery (61). Patients with essential thrombocythaemia, a disorder characterised by the high risk of thrombosis, have similarly increased MPA levels (46).

These MPA aggregates form when platelets are activated and undergo degranulation. Activated platelets express on their surface high levels of P-selectin that binds to the leukocyte receptor, P-selectin glycoprotein ligand-1 (PSGL-1) (62). In-vivo data confirms that P-selectin augments platelet-leukocyte aggregation (62). In experimental models, the infusion of recombinant human PSGL-1 reduced myocardial reperfusion injury in an animal model of vascular injury (63). Platelet-derived microparticles, which are shed from platelets upon their activation, also interact with monocytes in a mechanism dependent upon CD62P, similar to that seen with MPA formation (64). Additionally, platelet adhesion to monocytes may occur through calcium independent mechanisms, involving neither PSGL-1 nor P-selectin (65). Moreover, the soluble form of P-selectin may promote thrombogenic and proinflammatory transformation of leukocytes by stimulation of surface expression of receptors mediating their adhesive properties (e.g. integrins, Mac-1) or accelerating shedding of thrombogenic microparticles by leukocytes (66, 67). Thrombospondin is also reported to be involved in the cross-link between platelets and monocytes in early vascular injury via an interaction with glycoprotein IV on the surface of both cells (68). In fact, MPA is a good marker of platelet activation (e.g. in ACS) and superior to surface P-selectin expression as the latter is rapidly released into circulation by degranulated platelets whilst MPA continues to be detected in the circulation (69).

An important question relates to the biological role of MPA. They may simply reflect the physiological mechanism of elimination of activated platelets from the circulation and just represents a stage of platelet adherence to monocyte prior to their phagocytosis. As early as in 1966, Poole (70) demonstrated the phagocytosis of platelets by monocytes in organised arterial thrombi using electron microscopy. That means that high levels of MPA seen in ACS may perhaps reflect disproportionally high number of activated platelets in the circulation in relation to the
relatively small area of local vascular injury. Alternatively, monocytes may be attracted to remove excessive platelets.

However, the role of MPA might be even more complicated. It has been suggested that P-selectin may prime monocytes to increase platelet-activating factor synthesis, thus representing a vicious circle leading to progressive and uncontrolled platelet activation (71, 72). Additionally, conjugation with platelets activates expression of CD11b/CD18 (Mac-1) on monocytes, which amplifies interactions with platelets via fibrinogen bivalent linking Mac-1 with its platelet glycoprotein IIb/IIIa (73). The importance of MPA for the vascular thrombosis is further supported by the observation that monocytes constitute 16% of platelet thrombus-bound leukocytes, which represents an almost four-fold enrichment as compared with their proportion in circulating blood (74).

The conjugation of leukocytes with platelets is promoted by different conditions associated with inflammation and endothelial dysfunction (e.g. diabetes) (75, 76). Incubation of human blood with CRP doubles MPA formation and injection of LPS to mice increased MPA count four-fold (77). MPA – but not platelet aggregates with lymphocytes – and neutrophils are increased in patients with diabetes. Indeed, more severe diabetes (for example, complicated by proliferative retinopathy and nephropathy) is associated with an even higher number of MPA (76).

The increased association of monocytes with platelets accords with the data of enhanced of thromboxane A2 by monocytes in diabetic patients (78). The number of circulating MPA is increased with age and correlated with reduced synthesis of nitric oxide by platelets (79).

Not surprisingly, MPA formation is increased in patients with CAD (Table 2). Patients with stable CAD have increased circulating MPA compared with controls (15.3% vs. 6.3%, respectively) (80). However, destabilisation of atherosclerotic plaques complicated by atherothrombosis results in a profound (three-fold) increase in total and TF positive MPA found in blood of ACS patients compared to stable CAD (68, 81). For example, Furman et al. (82) examined 61 patients with acute myocardial infarction and compared them with 150 control subjects and reported that intensive formation of MPA in myocardial infarction starts very early and may be detected within four hours of symptom onset, even prior the increase in creatine kinase levels. However, utility of MPA count for diagnostic purposes in ACS has to be tested with direct comparison to cardiac troponins, and has important limitations relevant to a labour intensive procedure for its enumeration by flow cytometry and the lack of exclusive cardiac specificity.

Given the clear association of MPA with the onset of atherothrombosis, these may perhaps be appealing targets for antithrombotic treatment. However, only limited data are available on the effects of medical therapy on MPA levels. Of note, anticoagulation has little impact on monocyte-platelet interaction (83). In a recent randomised study of 60 patients undergoing percutaneous coronary intervention (the majority with stable CAD) anticoagulation with a combination of unfractioned heparin and eptifibatide was associated with higher amounts of MPA compared with bivalirudin, but this difference disappeared after pretreatment with clopidogrel (62). In one in-vitro study, the formation of platelet-leucocyte conjugates was markedly enhanced in the presence of glycoprotein IIb/IIIa antagonist, MK-852 (84). In contrast to anticoagulants and antiplatelet therapy reduce MPA (83, 85). This effect has been correlated with a concomitant decrease in monocyte expression of in Mac-1 ex-
Other mechanisms of monocyte prothrombotic effects

Participation of monocytes in the prothrombotic conditions is not restricted to the expression of TF and the generation of MPA. The evidence supports the impact of monocytes for thrombus generation, both directly via the secretion of procoagulant factors and indirectly, by promoting inflammation processes.

It is well known that inflammation affects the activity of various coagulation pathways. The inflammatory state also activates monocytes which possess receptors for CRP (for example, FcγRIIa [CD32]) and expresses CD40, a receptor for CD40 ligand (87–89). CRP and CD40 ligand induce TF expression in human monocytes, thus linking inflammation, coagulation and thrombosis (90–93). Stimulation of human monocytes/macrophages through CD40 by either membranes from activated T cells or recombinant CD40L further induces the expression of interstitial collagenase (MMP-1), stromelysin (MMP-3), recognised elements of plaque destabilisation and predictors of atherothrombotic events (37). Activated monocytes are able to bind and activate coagulation factor X responsible for the conversion of prothrombin to thrombin (94). Additionally, monocytes can bind coagulation factor V and also produces its activated form on their surfaces (95). Not surprisingly, patients with myocardial infarction were shown to have significantly higher monocyte procoagulant activity when compared to stable patients (96). To counterbalance their prothrombotic activity, resting monocytes produce thrombomodulin, an essential membrane cofactor of thrombin in the triggering of the natural anticoagulant protein C pathway; indeed, this production is significantly increased following monocyte activation (e.g. by LPS) (97).

Monocytes and thrombus recanalisation

So far, we have mainly discussed the deleterious prothrombotic effects of monocytes. However, monocytes also actively participate in antithrombotic processes (Fig. 1). These cells help eliminate thrombogenic activated platelets from the circulation (as discussed in the MPA section above). However, monocytes are crucial for thrombus recanalisation, and venous thrombi do often recanalise, so that patency of vascular lumen is restored (98).Arterial thrombi also recanalise, but perhaps less frequently and to a lesser extent.

Thrombus resolution involves several processes, as follows: (i) covering of the thrombus surface exposed to blood with monocytes and endothelial cells; (ii) penetration of monocytes inside the thrombus; (iii) thrombolysis and phagocytosis of thrombus components; (iv) generation of new capillaries in the thrombus (angiogenesis); and (v) in some instances, recanalisation – the restoration of the patient vessel (artery or veins) along the original vessel (99–101).

Histological studies have confirmed the formation of intra-thrombus clefts and neovascular channels that usually appear within the first four weeks (102). Monocyte chemotactant protein (MCP-1) is an important element of monocyte recruitment to the sites of thrombosis and is known to contribute to the organisation and resolution of venous thrombi (103). Deletion of CCR2 (MCP-1 receptor), at least in a mouse model, severely impairs DVT resolution (100, 104). In contrast, MCP-1 injected into thrombus increases thrombus organisation scores and reduces the thrombus area (105).

Attracted by high MCP-1 levels and other mechanisms (e.g. mediated by local inflammation) monocytes infiltrate the thrombus in large numbers and actively promote its recanalisation (106). Indeed, extracted peripheral blood monocytes generate tissue plasminogen activator, urokinase and the urokinase receptor (107–109). In fact, these cells are principle source of tissue plasminogen activator in the venous thrombus (106). Additionally, monocytes are capable of degrading fibrin even in the absence of plasmin (71). Fibrinolysis in venous thrombi is also mediated by monocyte-derived urokinase plasminogen activator, as evidenced from impaired thrombus resolution in mice lacking this enzyme (110, 111).

Restoration of venous blood flow is ultimately relies on thrombus neovascularisation with appearance of functional flow channels (112). A number of angiogenic cytokines and chemokines released by monocytes (e.g. vascular endothelial growth factor and basic fibroblast growth factor) are involved to the mobilisation of cells participating in the neovessel formation (e.g. endothelial progenitor cells) (113). The role of monocyte-dependent chemokine CXCR2 in the neovascularisation, collagen turnover, and fibrinolysis was further confirmed in an in vivo animal model of DVT (114). Of interest, although angiopoietin receptor Tie-2 is well known to be expressed on endothelial cells, it has recently been shown to be present on circulating monocytes (i.e. specifically on their CD14+/CD16- subset). Monocyte expression of Tie-2 is up-regulated by hypoxic environment, thus indicating their activation and enhanced potential of mobilisation to sites of local ischaemia (e.g. postthrombotic) and thrombus recanalisation (115).

Thus, monocytes and endothelial progenitor cells cooperate in restoration of blood flow in occluded thrombi. Moreover, a substantial part of endothelial progenitor cells themselves are constituted by monocyte-derived cells (116). Although monocytes are critically important for venous thrombus resolution they can also produce inhibitors of fibrinolysis, such as plasminogen activator inhibitors 1 and 2 and form TF, as discussed previously (117). It is therefore possible that the fibrinolytic/prothrombotic balance maintained by monocytes may determine the clinical outcome of venous thrombosis.

Conclusion

In addition to other blood cells, platelets monocytes are an important circulating cellular element of prothrombotic and anti-thrombotic mechanisms. In addition to their potential role as prognostic markers, monocytes might be an appealing target for future medical treatments in addition to more conventional management strategies. Indeed, the risk of bleeding complications is a major issue with new, novel antithrombotic medicines. As an alternative to platelets and coagulant factors, other directions...
should be considered by pharmaceutical companies developing new antithrombotic strategies. Being intimately involved in the modulation of thrombotic processes, monocytes have not as yet been compromised by an association with the risk of bleeding. Clearly, additional studies are warranted on this crossroad of haematology and cardiovascular medicine.

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