

Chapter Book

THROMBUS FORMATION ON DISRUPTED PLAQUES

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INTRODUCTION

Acute cardiovascular events that usually involve thrombus formation at sites of disrupted atherosclerotic plaques are currently described as atherothrombosis. Thrombosis is a major complication of atherosclerosis and also a rare but serious complication after stent implantation. However, it does not always result in complete thrombotic occlusion with subsequent acute symptomatic events (1). Therefore, thrombus growth is critical to the onset of clinical events. Thrombus formation is probably modulated by the thrombogenicity of exposed plaque constituents, local hemorheology, systemic thrombogenicity and fibrinolytic activity. Although the mechanisms of thrombus formation have been intensively investigated, little is known about either the mechanisms involved in thrombogenesis or thrombus growth after plaque disruption and stent implantation. This article examines the pathology of atherothrombosis, including late drug-eluting stent (DES) thrombosis, and recent advances in the understanding of thrombogenetic mechanisms and thrombus growth on atherosclerotic lesions, especially coronary atherothrombosis.

PATHOLOGY OF CORONARY ATHEROTHROMBOSIS

Arterial thrombi were traditionally considered to mainly comprise aggregated platelets because of rapid flow. However, recent evidence indicates that atherothrombi are composed of aggregated platelets, fibrin and other types of blood cells (2-4). Occlusive thrombi are consistently composed of platelets, fibrin and erythrocytes from the early phase of onset. Glycoprotein (GP) IIb/IIIa, a platelet integrin, intermingles with fibrin, and the surface of thrombi is mainly covered with GPIIb/IIIa and von Willebrand factor (VWF), a blood adhesion molecule that colocalizes with GPIIb/IIIa. Tissue factor (TF), an initiator of the coagulation cascade, is closely associated with fibrin (3). Tissue factor and/or VWF might contribute to thrombus growth and obstructive thrombus formation on disrupted atherosclerotic plaques.

Two major morphological features of plaque disruption are rupture and erosion (**Fig. 1**).

Plaque rupture is caused by disruption of the fibrous cap, which allows blood to come into contact with the thrombogenic necrotized core, resulting in thrombus formation. Plaque rupture likely occurs in plaques with a large necrotized lipid core and fibrous caps that are usually thinner than 65 μm and heavily infiltrated by macrophages, lymphocytes and occasional smooth muscle cells (SMC)s5. Plaque erosion is relatively superficial and probably occurs in plaques with thick fibrous caps with an abundance of SMCs and a proteoglycan matrix, especially when composed of versican and hyaluronan, but the necrotic core is small and often absent. Inflammatory cell infiltration is relatively low compared with plaque rupture (5). Angiographically, the diseased artery is less narrowed and irregular in erosion. Thrombi that develop on ruptured and on eroded plaques are fibrin- and platelet-rich, respectively. Both TF and C-reactive protein are abundant in ruptured, compared with eroded plaques (2). These distinct morphological features suggest

Figure 1. Microphotographs of human coronary plaque rupture and erosion. Upper: Ruptured plaque has a large necrotic core and disrupted thin fibrous cap associated with thrombus formation. Lower: Eroded plaque has SMC - and proteoglycan-rich lesion with thrombus. (From Sato et al.2, with permission).

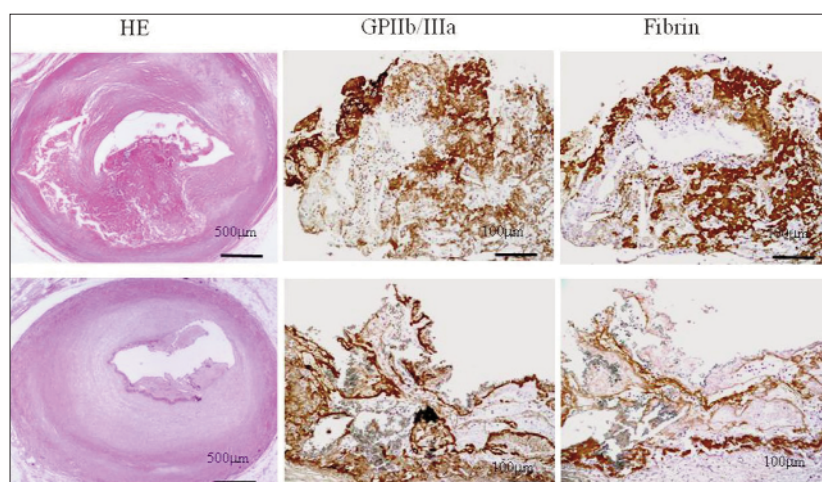
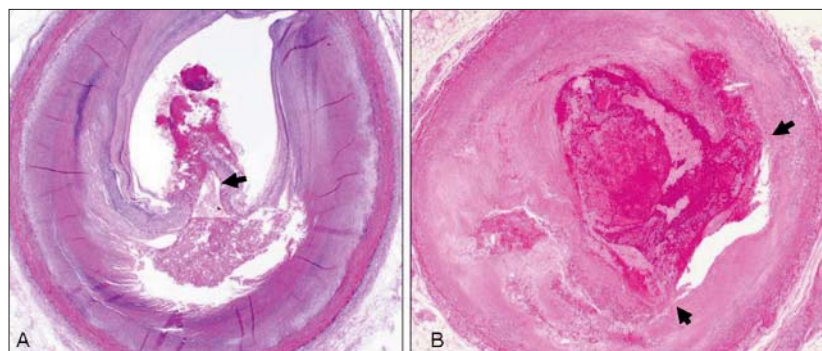


Figure 2. Microphotographs of human coronary thrombi in patients with non-cardiac death (A) and acute myocardial infarction. Atherosclerotic plaque rupture (arrows) does not result in thrombotic occlusion. Fibrous cap is disrupted and thrombus has formed in both arteries. However, thrombus size significantly differs. Thrombus from patients with non-cardiac death (A) is smaller and ruptured plaque has a smaller lipid core and mild stenosis, compared with those from patients with acute myocardial infarction (B).



that different mechanisms are involved in plaque rupture and erosion.

On the other hand, the disruption of atherosclerotic plaques does not always result in complete thrombotic occlusion with subsequent acute symptomatic events (**Fig. 2**).

Clinical studies using angiography and intravascular ultrasound have revealed that multiple plaque rupture is a frequent complication in patients with coronary atherothrombosis (6). Healed stages of plaque disruption are also occasionally observed in autopsy cases with or without coronary atherothrombosis (7). To evaluate the incidence and morphological characteristics of thrombi and plaque disruption in patients with non-cardiac death, Sato et al. (8) examined 102 hearts from non-cardiac death autopsy cases and 19 from patients who died of acute myocardial infarction (AMI). Surprisingly, they found coronary thrombi in 16% of the cases with non-cardiac death, and most of them developed on eroded plaques. However, the thrombi were too small to affect the coronary lumen (**Table 1**).

The disrupted plaques from the patients who died of non-cardiac causes had smaller lipid areas, thicker fibrous caps and more modest luminal narrowing than those who died of AMI. These findings suggest that the onset of acute coronary events represents the tip of the atherothrombosis iceberg and that regional factors influence coronary thrombus growth after plaque disruption.

Table 1. Incidence of thrombosis in NCD and AMI

	NCD (n = 102)	AMI (n = 19)	p value
Fresh thrombus	10 case (10%)	14 case (75%)	<0.001
- Erosion	7 case (7%)	4 case (21%)	0.07
- Rupture	3 case (3%)	10 cases (54%)	<0.001
Old thrombus	6 case (6%)	5 cases (26%)	<0.05

NCD, non-cardiac death; AMI, acute myocardial infarction (From Sato et al.8, with permission)

PATHOLOGY OF LATE DRUG-ELUTING STENT THROMBOSIS

Coronary drug-eluting stent (DES) implantation has become the treatment of choice for patients with symptomatic coronary artery disease undergoing percutaneous coronary revascularization. Although DES have dramatically reduced rates of restenosis compared with bare metal stents, late thrombosis has emerged as a major concern (9). Premature discontinuation of antiplatelet therapy, renal failure and diabetes mellitus are clinical predictors of DES thrombosis. However, the pathogenesis of late/very late DES thrombosis remains obscure. **Figure 3** shows immunofluorescent and immunohistochemical images of coronary late DES thrombus (10).

All DES thrombi are composed of aggregated platelets and fibrin (**Fig. 3** upper), together with erythrocytes and white blood cells, and they were all constitutively immunopositive for GPIIb/IIIa, fibrin, glycophorin A (a membrane protein expressed on erythrocytes), P2Y12 receptor (a pyrinergic receptor targeted by thienopyridine), VWF and CD16 (a marker of neutrophils). The proportions of these factors and cells did not significantly differ from those of de novo coronary thrombi associated with AMI (**Table 2**).

The P2Y12 receptor colocalized with GPIIb/IIIa (**Fig. 3** middle), which supports the clinical fact that intensive anti-platelet therapy with thienopyridine prevents DES thrombosis. Because cytochrome p-450 influences the response to clopidogrel, the novel thienopyridine prasugrel might be more suitable for preventing DES thrombosis, as well as de novo AMI. Interestingly, the shape of the CD34-positive cells in thrombi is heterogeneous (**Fig. 3** lower), which could reflect differentiation or a different origin, and might participate in the organizing process of thrombi. Because CD34-positive endothelial progenitor cells can bind and inhibit platelet function and thrombus formation (11), capturing the cells on struts or in thrombi might protect thrombus growth (12).

The mechanisms of late/very late DES thrombosis are poorly understood. Angioscopic and pathological studies have revealed that delayed neointimal covering is associated with DES thrombosis (13,14). In contrast, a serial analysis with optical coherence tomography demonstrated neointimal coverage between 6 and 12 months after DES implantation (15).

Drug-eluting stents might induce a local hypersensitivity reaction with positive coronary remodelling, resulting in incomplete stent apposition (16). Vascular remodelling positively correlates with eosinophil counts in coronary thrombi. However, the role of eosinophils in late DES thrombosis is controversial, because eosinophils were found in only half of the late/very late DES thrombi as a minor component (**Table 2**).

Figure 3. Representative immunofluorescent and immunohistochemical images of very late drug-eluting stent (DES) thrombus. Upper and middle: Images stained with fluorescein isothiocyanate-labeled GPIIb/IIIa (green), Cy3-labeled fibrin or P2Y12 (red), and merged immunofluorescent images. Areas with colocalized factors are stained yellow. Lower: Oval CD34-positive cells in clusters (left) and flat CD34-positive cells covering DES thrombus at organizing stage (left). (From Nishihira et al. 10, with permission).

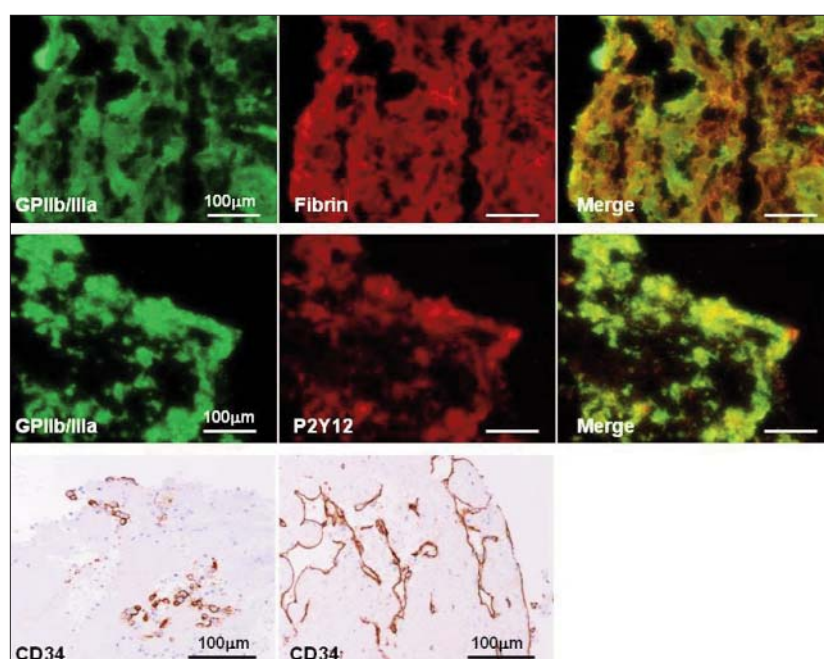


Table 2. Immunopositive area or cell counts in late/very late DES thrombus and de novo AMI thrombus

	de novo AMI (n = 29) median; range	late/very late DES (n = 9) median; range	p value
GPIIb/IIIa (%)	17.2; 7.2–31.4	17.0; 6.6–30.3	0.46
Fibrin (%)	30.1; 4.2–50.2	36.8; 8.2–77.2	0.16
Glycophorin A (%)	22.3; 0.2–51.5	25.6; 0.2–43.3	0.62
P2Y12 (%)	20.5; 0.8–48.1	16.4; 8.9–32.2	0.57
VWF (%)	11.9; 1.1–25.2	12.2; 1.3–38	0.68
CD16 (/mm ²)	877; 133–2428	487; 217–2887	0.37
CD34 (/mm ²)	4; 0–44.3	18.2; 0–530	0.34
Eosinophil (/mm ²)	1.2; 0–28.2	0.6; 0–3.6	0.28

DES, drug-eluting stent, AMI, acute myocardial infarction, GP, glycoprotein, VWF, von Willebrand factor

MECHANISMS OF PLAQUE RUPTURE

Thinning and disruption of the fibrous cap caused by metalloproteases is likely to be involved together with local rheological forces and emotional status. Accumulating evidence supports the notion that inflammation plays a key role in the pathogenesis of plaque rupture.

The numerous inflammatory cells in rupture-prone atherosclerotic plaques can produce enzymes that degrade the extracellular matrix of the fibrous cap. Macrophages in human atheromas overexpress interstitial collagenases, gelatinases and elastolytic enzymes.

Activated T lymphocytes and macrophages can secrete interferon γ (INF- γ), which inhibits collagen synthesis and induces the apoptotic death of SMC (17). Moreover, INF- γ can induce interleukin (IL)-18, which accelerates inflammation. Interleukin-18 colocalizes with INF- γ in macrophages located at the shoulder region, but not at the necrotic core, and it is associated with coronary thrombus formation in patients with ischemic heart disease (18).

Interestingly, the important anti-inflammatory cytokine IL-10 is also upregulated in macrophages in atherosclerotic lesions from patients with unstable, compared with stable angina (19). The heterogeneity of macrophages in atherosclerotic plaque could explain these paradoxical findings (20). This evidence indicates that an imbalance of the inflammatory pathway appears to participate in the plaque destabilization that triggers thrombosis in fibrous cap rupture.

Intraplaque hemorrhage might also trigger plaque rupture. The frequency of previous hemorrhages is greater in coronary atherosclerotic lesions with late necrosis and with a thin fibrous cap than in those with early necrosis or intimal thickening (21). Intraplaque hemorrhage and iron deposition are more prominent in coronary culprit lesions obtained by directional coronary atherectomy from patients with unstable, than with stable angina pectoris. The iron deposition correlated with oxidized low density lipoprotein and thioredoxin, an anti-oxidant protein, and was also associated with thrombus formation (22). The pathological findings imply relationships among intraplaque hemorrhage, oxidative stress and plaque instability. However, direct evidence linking intraplaque hemorrhage and plaque instability remains elusive.

MECHANISMS OF PLAQUE EROSION

Some authors have postulated that erosions result from vasospasm, but the mechanisms of plaque erosion remain poorly understood. About 80% thrombi of plaque erosions are non-occlusive despite sudden coronary death (5). Platelet-rich emboli are found in 74% of patients with plaque erosion who suddenly die, which is an event that is more frequently associated with plaque rupture (40%). Because activated platelets release vasoconstrictive agents such as 5-hydroxytryptamine (5-HT, serotonin) and thromboxane A₂, these emboli might increase peripheral resistance leading to altered coronary blood flow. Furthermore, 5-HT can induce the hypervasoconstriction of SMC-rich atherosclerotic vessels via 5-HT_{2A} receptors and reduce blood flow during thrombogenesis (23,24). Endothelial cells preferentially undergo

apoptosis at areas of atherosclerotic plaques downstream of a blood flow disturbance and shear stress is lower than at upstream areas (25). Experimental aortic stenosis can induce acute endothelial change or damage the normal aorta (26). Therefore, hemodynamic forces, particularly blood flow disturbed by stenosis or vasoconstriction, could be a crucial factor in generating surface vascular damage and thrombosis. Actually, blood flow disturbed by acute vascular narrowing induces superficial erosive damage to the SMC-rich neointima at post stenotic regions in rabbit femoral arteries. **Figure 4** shows microscope images of a longitudinal section of the neointima at the post-stenotic region at 15 min after vascular narrowing.

Endothelial cells and SMCs at this region were broadly detached, and associated with platelet adhesion to the sub-endothelium. The superficial erosive damage also induced the apoptosis of endothelial cells and superficial SMCs within 15 minutes (27). Thus, disturbed blood flow can induce superficial erosive damage to SMC-rich plaque and thrombus formation at post stenotic regions. Computational fluid simulation analysis has indicated that oscillatory shear stress contributes to the development of erosive damage to the neointima (27).

Although direct clinical evidence has not yet supported the notion that coronary artery vasospasm plays a significant role in plaque erosion, the histological features of this erosive damage caused by disturbed blood flow is similar to those of human plaque erosion (2,5). Also, platelet and blood coagulation in the coronary circulation is activated after vasospastic angina (28).

Therefore, this evidence suggests that an acute-onset disturbed blood flow due to vasoconstriction could trigger plaque erosion. Hemodynamic factors could play an important role in the development of plaque erosion.

COAGULATION FACTORS INVOLVED IN THROMBUS GROWTH

The most fundamental difference between normal and atherosclerotic arteries is that active TF is abundant in atherosclerotic lesions (29). Therefore, vascular wall TF might contribute to thrombus formation /growth on atherosclerotic lesions. However, recent studies have detected low levels of TF in the blood that are nevertheless sufficient to support clot formation *in vitro*. Plasma TF levels are elevated in patients with unstable angina and AMI, and they correlate with adverse outcomes (30). Therefore, whether or not vascular wall and/or blood-derived TF supports thrombus propagation remains controversial.

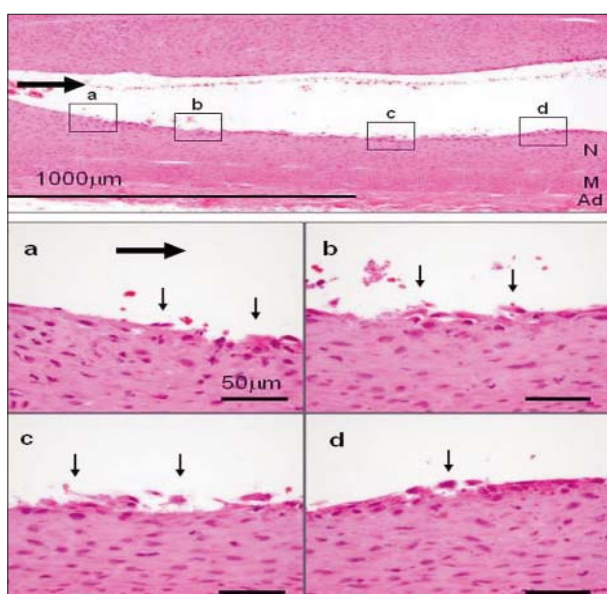


Figure 4A. Representative longitudinal microphotographs of rabbit femoral artery with smooth muscle cell-rich neointima 15 minutes after vascular narrowing. Broad superficial neointimal damage at post stenotic region. Neointimal cell detachment (small arrows) at high magnification. N, neointima; M, media; Ad, adventitia.

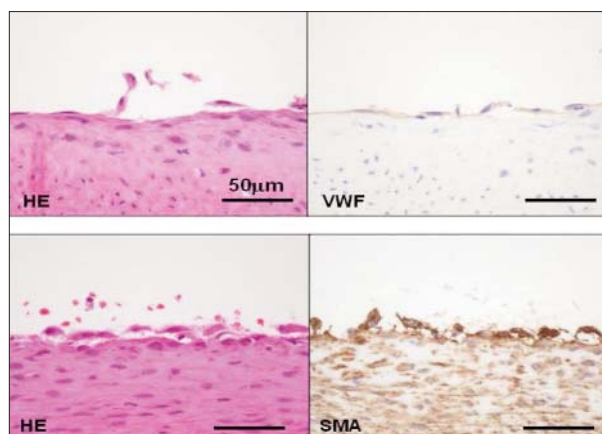


Figure 4B. Representative longitudinal microphotographs of rabbit femoral artery with smooth muscle cell-rich neointima 15 minutes after vascular narrowing. Immunohistochemistry to detect von Willebrand factor (VWF: endothelial marker) and smooth muscle actin (SMA: smooth muscle marker) confirmed detachment of endothelial and smooth muscle cells at post stenotic region. HE (hematoxylin eosin) (From Sumi et al.27)

Hematopoietic cell-derived, TF-positive microparticles contribute to laser injury-induced thrombosis in the microvasculature of the mouse cremaster muscle (31). In contrast, hematopoietic cell-derived TF does not contribute to photochemically-induced thrombosis in the mouse carotid artery (32). Yamashita et al. (33,34) demonstrated quantitative and qualitative differences between thrombi on atherosclerotic and normal arteries, and they found that TF derived from the vascular wall rather than from blood significantly contributes to thrombus formation/growth on atherosclerotic lesions. Atherosclerotic lesions express TF and have more procoagulant activity compared with the normal femoral artery (Fig. 5A).

Balloon injury to the neointima induced large platelet-fibrin thrombi, whereas such injury to normal femoral arteries induced small platelet thrombi (Fig. 5B) (33,34).

Thrombin generation mediated by neointimal TF contributed to the enhanced platelet aggregation and fibrin formation in atherosclerotic, but not in normal arteries. Moreover, whole blood coagulation in the model was not affected by inhibiting blood TF using a TF antibody even under hyperlipidemic conditions (34). Therefore, atherosclerotic plaque-derived TF might contribute to activation of the intravascular coagulation cascade and thrombus growth on atherosclerotic lesions.

Recent *in vitro* studies have shown that various types of blood cells, including monocytes, neutrophils, eosinophils and even platelets, can synthesize TF. Although TF expression in these blood cells is a matter of debate, monocytes are probably the only blood cells that synthesize and express TF, and activated platelets play a role in decrypting monocyte TF activity in a process entailing TF transfer to activated platelets (35). A related topic is the contribution of microparticles (MPs) to thrombus formation. Microparticles are small fragments of membrane-bound cytoplasm that are shed from the surface of activated or apoptotic cells. The procoagulant activity of MPs increases with exposure to phosphatidylserine and the presence of TF. Leroyer et al. (36) showed that MPs are more abundant and more thrombogenic in human atherosclerotic plaques than in plasma. Most MPs from plaques originate from macrophages, erythrocytes,

Figure 5A. Immunohistochemical and immunofluorescent images of rabbit femoral arteries. Representative immunohistochemical microphotographs of normal femoral artery and of femoral artery at 3 weeks after balloon injury under 0.5% cholesterol diet. Both 0.5% cholesterol diet and balloon injury induced atherosclerotic neointima, which is composed of smooth muscle cells (SMC) and macrophages. Neointimal cells express tissue factor (TF). I, intima; M, media; Ad, adventitia. HE/VB, hematoxylin eosin/Victoria blue stain.

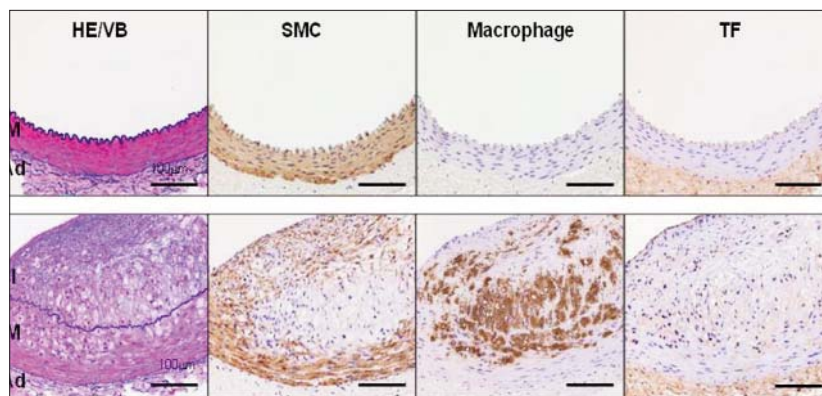
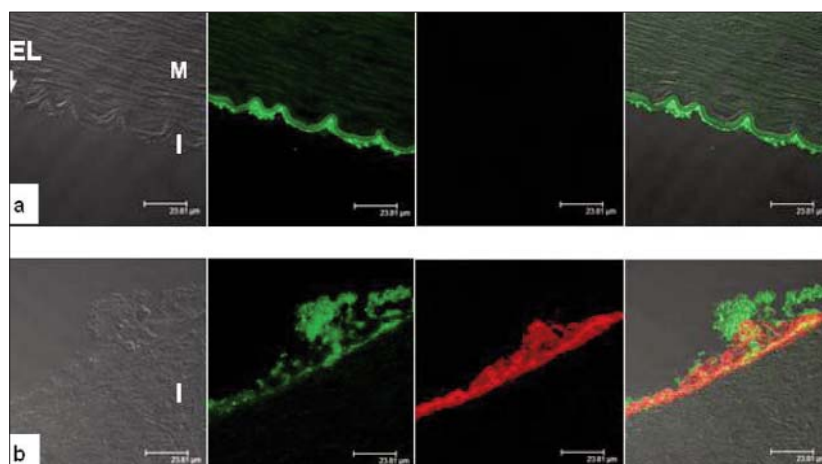


Figure 5B. Immunohistochemical and immunofluorescent images of rabbit femoral arteries. Representative immunofluorescent microphotographs of thrombi 15 minutes after balloon injury of normal femoral artery and of atherosclerotic neointima under 0.5% cholesterol diet. Rows show differential interference contrast images, fluorescein isothiocyanate-labeled GPIIb/IIIa (green) and Cy3-labeled fibrin (red) images, as well as merged immunofluorescent images. Areas with colocalized factors are stained yellow. Thrombi on normal intima are composed of small aggregated platelets (a), while those on atherosclerotic neointima are large, and composed of platelet and fibrin (b). I, intima; M, media; Ad, IEL, internal elastic lamina. (From Yamashita et al.34, with permission).



SMCs and lymphocytes. Both plaque and plasma MPs express TF and generate thrombin, but their coagulation activity is twice as high when isolated from plaques. Thus, MPs might play an important role in thrombus growth after plaque disruption. In fact, MPs are significantly elevated in acute coronary syndrome and ischemic strokes (37,38). However, whether elevated levels of MPs are a cause or a consequence of atherothrombosis remains unclear. Future studies are required to clarify the contribution of blood-derived TF and/or MPs to thrombus propagation on atherosclerotic lesions.

Another mechanism that might contribute to thrombus propagation *in vivo* is the intrinsic pathway of coagulation, which is initiated when coagulation factor XII (FXII) comes into contact with negatively charged surfaces in a reaction involving the plasma proteins, high molecular mass kininogen and plasma kallikrein. In contrast to TF, FXII is likely to become activated on the negatively charged surfaces of activated platelets. Factor XI (XI) is activated by activated FXII, thrombin, and activated XI. Feedback activation of FXI by thrombin promotes further thrombin generation *in vitro* (39). Studies *in vivo* have revealed the significance of the intrinsic coagulation pathway in thrombus formation. Initial platelet adhesion and aggregation are normal in mice lacking FXII and FXI, but thrombus propagation is defective in the damaged aorta, carotid artery and mesenteric arterioles (40). Mice deficient in FXII are more resistant to thrombosis than those deficient in FXI or Factor IX, indicating that FXII and FXI function via indistinct pathways (41). FXI is present in platelet-fibrin thrombus-induced balloon injury of the rabbit atherosclerotic neointima, and anti-FXI antibody reduces thrombus growth without prolonging bleeding time (42). Intrinsic coagulation factors play an important role in thrombus growth via further thrombin generation.

ROLE OF BLOOD FLOW IN THROMBUS GROWTH

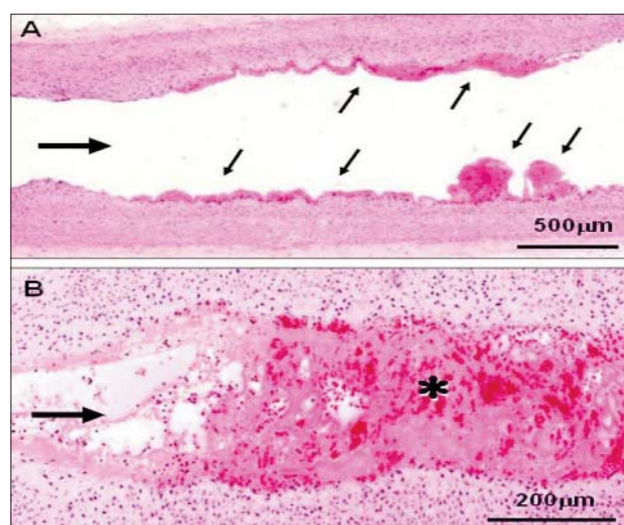
Blood flow is surely a key modulator of thrombus growth. Clinical studies have shown that blood flow is altered during coronary atherothrombosis and intervention. Marzilli et al. (43) reported an approximate 80% reduction in coronary blood flow during ischemia in patients with unstable angina. Hearts obtained from patients after sudden coronary death contained a mean of 4.5 intramyocardial microemboli, which are more common in plaque erosion than rupture and are associated with myocardial necrosis. Most (89%) affected microvessels were < 120 μm in diameter (44). Not only spontaneous plaque rupture and ulceration, but also coronary intervention can induce microembolization that leads to a reduction in coronary flow reserve (45). The mechanisms are considered to be largely responsible for the rapid elevation of distal vascular resistance due to microvascular embolism and vasoconstriction (46). However, how blood flow affects thrombus growth on atherosclerotic lesions remains obscure.

Yamashita et al. (33) demonstrated the effect of blood flow on thrombus propagation in diseased arteries of rabbits. Platelet-fibrin mural thrombi on atherosclerotic neointima became occlusive in the setting of a blood flow reduction of > 75%. In contrast, small platelet thrombi on normal arteries did not grow even under a 90% reduction in blood flow. Increased thrombogenicity of the vascular wall would be essential to platelet-fibrin thrombus formation, and changes in blood flow could be crucial for thrombus growth.

In addition to distal vascular resistance, disturbed blood flow induced by acute vascular narrowing promotes thrombus growth at post-stenotic regions. As described above, vascular narrowing of the rabbit femoral artery caused a superficial erosive injury at post stenotic regions of SMC-rich neointima (**Fig. 4A**). Mural thrombi became occlusive in 3 of 5 arteries after 3 hours (**Fig. 6**) and detached SMCs were involved in the thrombus.

The intimal injury significantly progressed 3 h after vascular narrowing. The thrombi that developed on the neointima consisted of a mixture of aggregated platelets and a considerable amount of fibrin. In contrast, the identical vascular narrowing of normal femoral arteries also induced endothelial detachment with small platelet thrombi at post stenotic regions, but fibrin and occlusive thrombi did not develop. Neither vascular narrowing nor anti-rabbit TF antibody affected whole blood hemostatic parameters in the rabbits, and no fibrin was generated in thrombi on eroded normal intima (27). The evidence indicates that TF derived from eroded neointima plays a crucial role in fibrin formation and thrombus growth rather

Figure 6. Thrombus growth at post stenotic region of rabbit femoral artery. Mural thrombus formation (small arrows; A) and occlusive thrombus formation (asterisk; B) on smooth muscle cell rich neointima 30 minutes after vascular narrowing. Large arrows indicate flow direction (From Sumi et al.27).



than circulating TF. Therefore, flow that becomes disordered after plaque disruption might contribute to thrombus growth in both distal resistance and proximal stenosis. The rheological effect on thrombus growth might be partly explained by a shear gradient-dependent platelet aggregation mechanism. Generally, soluble agonists generated at sites of vascular injury are thought to initiate platelet aggregation and thrombus growth. However, platelets can form aggregates *in vivo* without detectably increasing cytosolic calcium or undergoing a shape change and without substantial α -granule secretion during the early phases of thrombus development (47,48). These facts have raised the notion that additional mechanisms are involved. Using stenotic microvessels *in vitro* and *in vivo* along with imaging systems, Nesbitt et al. (49) revealed a shear gradient-dependent platelet aggregation process that is preceded by soluble agonist-dependent aggregation. A shear microgradient at post stenotic regions or at the downstream face of thrombi induces stable discoid platelet aggregates with restricted tethers, and the magnitude and spatial distribution of shear microgradients directly influence the size of platelet aggregation. This process required ligand binding to integrin $\alpha_{IIb}\beta_3$ and an inositol triphosphate - and extracellular Ca^{2+} - dependent transient Ca^{2+} flux, but not global platelet shape, degranulation or soluble agonists. Stabilized discoid platelet aggregates were consolidated within the thrombus base, and depended on soluble agonists.

These findings suggest that platelets principally use a biomechanical platelet aggregation mechanism to promote the accumulation and stabilization of discoid platelets at sites of vascular injury. Vessel and/or thrombus geometry itself might promote thrombus formation.

PLATELET RECRUITMENT IN THROMBUS GROWTH

Adhesion molecules and their receptors on platelets are essential for thrombus formation because these molecules support platelet tethering, tight adhesion, aggregation and platelet recruitment to the thrombus surface. The crucial importance of platelet adhesion receptors such as GPIb α or the GPIIb/IIIa has been confirmed in gene-targeted mice. The absence of these receptors leads to highly impaired thrombus formation, regardless of the model in which vascular injury is induced (50). The large, multimeric, plasma protein VWF undergoes a conformational change when bound to a matrix, a process that allow VWF to bind GPIb α . Recent experimental evidence *in vitro* and *in vivo* has shown that platelet recruitment on the thrombus surface is primarily mediated by VWF and GPIb α on flowing platelets (51,52). The VWF-GPIb α interaction is crucial in both the initial step of platelet adhesion to the injured vessel wall and in the recruitment of new platelets to growing thrombus. However, the roles of VWF on fibrin-rich atherothrombus are limited. Its presence in human coronary thrombi suggests that VWF plays an important role in this process (3,4). A monoclonal antibody against the VWF A1 domain, which interacts with platelet GPIb α significantly inhibited the formation of fibrin-rich mural thrombus induced by balloon injury of rabbit

atherosclerotic neointima (53). Moreover, the antibody completely inhibited occlusive thrombus formation on rabbit atherosclerotic vessels even when blood flow was disturbed (33). Thus, VWF plays an important role in thrombus propagation on atherosclerotic lesions via platelet recruitment.

The size of the VWF multimer can affect thrombus size and it is regulated by a plasma protease, a disintegrin and metalloprotease with a thrombospondin type 1 motif 13 (ADAMTS-13). Hepatic satellite cells synthesize and secrete ADAMTS-13, and its deficiency leads to an increased level of massive circulating VWF multimers, and correlates with the onset of the general thrombotic disease, thrombotic thrombocytopenic purpura (TTP). Surprisingly, mice deficient in ADAMTS-13 are viable with no apparent signs of TTP, but they are susceptible to thrombus formation in small vessels and in the pulmonary circulation (54,55). These findings suggest that ADAMTS-13 modulates thrombus formation in various vascular beds and regulates thrombus growth in atherosclerotic arteries. Clinical evidence suggests that the size of the VWF multimer is dysregulated in patients with AMI. The ratio of VWF/ADAMTS-13 antigen is higher in patients with AMI than in those with stable angina pectoris, and the correlation between plasma VWF antigen and ADAMTS-13 activity is inverse in AMI patients (56). ADAMTS-13 closely localizes with VWF in fresh coronary thrombi from patients with AMI (57). Reducing ADAMTS-13 activity using a monoclonal antibody against the disintegrin-like domain enhances platelet thrombus growth on immobilized type I collagen at a high shear rate (1500S-1) and platelet-fibrin thrombus formation on the injured neointima of rabbit femoral arteries (57). That study also showed that massive VWF multimers are cleaved during platelet thrombus formation under a high shear rate. The site of VWF-cleavage by ADAMTS-13 is localized to the surface of platelet thrombus, and ADAMTS-13 activity is shear-dependent (58). Thus, ADAMTS-13 may work at the site of ongoing thrombus generation and limit thrombus growth.

Other possible factors that enhance thrombus growth on atherosclerotic vessels are molecules that affect thrombus stabilization and platelet potentiation. Activated platelets release adenosine diphosphate (ADP), 5-HT, and thromboxane A₂, which further promotes activation. This self-amplifying process will lead to thrombus stabilization. ADP-receptor mediated cyclic calcium signaling requires sustained GPIIb/IIIa activation and thrombus stabilization *in vitro* (59). Ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) 1 is a cell membrane protein that rapidly hydrolyzes both ADP and adenosine triphosphate to adenosine monophosphate and thereby inhibits platelet aggregation on endothelial cells and SMCs (60). The expression of E-NTPDase in atherosclerotic lesions can be down-regulated in patients with unstable angina (61) and vascular wall E-NTPDase modulates thrombus formation and vascular contraction in rat carotid arteries (60,62). These findings suggest that reduced E-NTPDase activity in atherosclerotic lesions affects thrombus stabilization and promotes thrombus propagation on atherosclerotic lesions. However, direct evidence of a correlation between E-NTPDase activity and thrombus size remains elusive.

CONCLUSIONS

Recent evidence indicates that inflammation or altered blood flow plays a key role in the pathogenesis of plaque rupture and erosion, respectively. Occlusive atherothrombus is consistently composed of aggregated platelets, fibrin, erythrocytes, and neutrophils. The enhanced platelet aggregation and fibrin formation in human coronary artery and rabbit atherosclerotic artery indicates excess thrombin generation mediated primarily by plaque TF and a subsequent intrinsic coagulation pathway. Oscillation of shear stress and the shear gradient by vessel and/or thrombus geometry can initiate superficial erosive injury and the early phase of platelet aggregation. The role of blood cell-derived TF and MPs in thrombus formation after plaque disruption is currently unclear, and further studies are required to determine whether or not these factors support thrombus growth. Appropriate models of atherothrombus, but not of thrombus in the normal artery, can provide valuable information about their pathophysiological roles. Moreover, the mechanisms of late DES thrombosis remain unknown. The similar content between late/very late DES thrombus and AMI thrombus *de novo* implies excess thrombin generation and altered blood flow in thrombus formation after DES implantation.

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