Role of Thrombogenic Factors in the Development of Atherosclerosis

Kousuke Marutsuka¹, Kinta Hatakeyama², Atsushi Yamashita², and Yujiro Asada²

¹ Pathology Division, Miyazaki Medical College Hospital, ² First Department of Pathology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan.

Hemostatic factors play a crucial role in generating thrombotic plugs at sites of vascular damage (atherothrombosis). However, whether hemostatic factors contribute directly or indirectly to the pathogenesis of atherosclerosis remains uncertain. Autopsy studies have revealed that intimal thickening represents the first stage of atherosclerosis and that lipid-rich plaque arises from such lesions. Several factors contribute to the start of intimal thickening. Platelets release several growth factors and bioactive agents that play a central role in development of not only thrombus but also of intimal thickening. We have been investigating which coagulation factors simultaneously, or subsequently with platelet aggregation, participate in thrombus formation. Tissue factor (TF) is an essential initiator of blood coagulation that is expressed in various stages of atherosclerotic lesions in humans and other animals. Factors including thrombin and fibrin, which are downstream of the coagulation cascade activated by TF, also contribute to atherosclerosis. TF is involved in cell migration, embryogenesis and angiogenesis. Thus TF, in addition to factors downstream of the coagulation cascade and the protease-activated receptor 2 activation system, would be a multifactorial regulator of atherogenesis. J Atheroscler Thromb, 2005; 12: 1–8.

Key words: Thrombotic factors, Atherogenesis, Tissue factor, PAR2

Introduction

Many hypotheses have attempted to explain the multifactorial pathogenesis of atherosclerosis (1–3). Thrombus formation is important in cardiovascular diseases (4) including atherogenesis and atherothrombosis, as it results in cardiovascular events. Some prospective studies have shown that increased plasma levels of fibrinogen, as well as of factors VII and VIII indicate a risk of cardiac events (5).

However, whether hemostatic factors contribute directly or indirectly to the pathogenesis of atherosclerosis remains uncertain. For example, hypercoagulable states, such as an antithrombin deficiency and Factor V Leiden, generally predispose individuals to venous thrombotic events, but not to atherosclerosis (6). Furthermore, hemophilia states, such as a factor VIII deficiency or von Willebrand’s disease, do not seem to protect against atherosclerosis (7), although the opposite finding has been reported (8).

Such controversial evidence complicates the understanding of the precise mechanisms of atherogenesis. Autopsy studies have frequently shown that the structure of atherosclerotic lesions is multilayered (Fig. 1) with occasional persistent layers of fibrin (Fig. 2). These findings suggest that repeated thrombus formation and organization contribute to the development of atherosclerosis. We and others have demonstrated that tissue factor (TF), an essential initiator of the coagulation cascade, is expressed during various stages of atherosclerotic lesions. TF is an important determinant of thrombogenicity that contributes to fibrin-rich thrombus formation after plaque disruption and to the progression of atherosclerotic lesions (9–11). Furthermore, TF might play roles in processes other than coagulation, such as embryogenesis or angiogenesis (12) and cell migration (13).
Other factors that contribute to atherogenesis include the fibrinolytic system and lipoprotein (a) [Lp (a)]. The fibrinolytic system is important in thrombus resolution at sites not only of injured vessels but also of atherosclerotic plaque rupture. Low levels of plasma fibrinolytic factors might be important in predicting atherothrombotic events (14–16). In fact, alterations in plasma fibrinolytic factors might be a consequence of atherosclerosis. Many prospective studies have found an unadjusted positive association between plasma fibrinolytic markers (e.g., tissue plasminogen activator antigen or plasminogen activator inhibitor antigen) and risk of cardiovascular disease (17). The association tends to be strong, but it is often rendered statistically insignificant after adjustment for other risk factors. Some genetic epidemiological studies have suggested that polymorphism of a gene coding for fibrinolysis is associated with cardiovascular disease, but that the relative risk is modest (18). Thus, current epidemiological evidence regarding whether impaired fibrinolysis is a “cause” of atherothrombotic events is inconclusive. Elevated levels of Lp (a) are closely linked to the onset of cardiovascular disease and since Lp (a) appears to inhibit natural fibrinolytic activity, it might represent an important link between thrombotic and lipid atherogenic mechanisms (19).

Thrombus formation depends on a balance between thrombotic and fibrinolytic abilities, therefore, excessive activation of the thrombotic pathway and an impaired fibrinolytic system can cause a predisposition to atherosclerosis and atherothrombosis (14, 20). This review focuses on the contribution of the key components of hemostasis, especially the blood coagulation system, TF and the protease-activated receptor 2 (PAR2) system, to atherogenesis.

Histological classification of atherosclerosis

Although this review does not address atherosclerosis in detail, an understanding of the various morphological types of plaque is essential to understanding atherogenesis.

The current model has established the following consecutive stages in the development of atherosclerosis (21, 22). (a) A lesion is initiated and characterized by the inflammatory leukocyte recruitment by activated endothelial cells and extracellular lipid accumulation in the intima. (b) Smooth muscle cell (SMC) migration and proliferation is accompanied by the synthesis of extracellular matrix and the transformation of recruited macrophages to lipid-rich foam cells due to the accumulation of oxidized low-density lipoprotein (oxLDL). (c) Lesion progression is characterized by metalloprotease degradation of the matrix and by weakening of the fibrous cap, and then (d) plaques rupture and thrombus forms at the site of the lesion. Thrombus occasionally forms at an erosive atherosclerotic plaque without breakdown of the plaque fibrous cap. According to these concepts, the American Heart Association (AHA) established a task force (23–25) that introduced a standard nomenclature for plaque ranging from I to VI. Monocyte migration and fatty streaks characterize Types I and II, respectively, as described in (a). Small pools of extracellular lipid appear in Type III, as described in (b). These lipid deposits then coalesce to form a larger core of extracellular lipid. Thereafter, SMC migration and proliferation encapsulate the lipid core in collagen, culminating in the advanced fibrolipid, fibroatheromatous, Types IV and V plaque, described as (c) and (d), respectively.

Autopsy studies of individuals who died of non-cardio-
vascular diseases have revealed intimal lesions in individuals up to 5 years of age that gradually progress with age even among teens (26–28). In adults over 50 years of age, the aorta can contain all types of lesions at any stage. This implies that plaque is formed throughout life (29). We have also attempted to evaluate and classify the progression of intimal thickening in the coronary arteries of Japanese youth (30). Intimal lesions initiated from the time of childhood, gradually thickened with or without fat deposition. Stenotic lesions developed until late middle age, about 40% of which consisted of fibrous thickening without fatty plaque. This intimal fibrous thickening form of atherosclerosis does not appear in the AHA classification. These findings indicate that fibrous lesions constitute the basal changes in atherosclerosis.

New concept of coagulation cascade

Hoffman and Moore have proposed a new concept of the coagulation cascade (31) (Fig. 3). Biological evidence from the fluid phase and the pericellular progression of the coagulation cascade is simple to understand, especially with respect to a mild hemorrhagic tendency among hemophiliacs and efficient hemostasis in blood vessel walls.

The classical model of coagulation is that of a cascade involving the activation of various clotting factors along either an extrinsic or an intrinsic pathway. According to this model, stimulation of either of these pathways can result in the production of a large amount of thrombin and subsequent formation of a fibrin clot (32). Although this cascade paradigm supports the results of experimental evaluations of coagulation disorders and demonstrates interactions between coagulation factors, it does not adequately explain the pathophysiological mechanisms of the hemostatic system. In particular, the model does not explain why some patients have a hemorrhagic tendency or how it can accurately predict which patients will actually bleed. For instance, patients who are deficient in factor XII (FXII), high-molecular-weight kininogen, or prekallikrein do not present with a bleeding tendency despite a prolonged partial thromboplastin time (PTT), which indicates a functional disturbance of the intrinsic pathway (31). In contrast, predisposition to hemorrhagic risk might be increased among patients who are deficient in FXI. The length of the prolonged PTT in this disorder, however, does not necessarily predict the extent of a bleeding tendency, which is less severe than that associated with hemophilia. The cascade hypothesis cannot account for the varying degrees of hemorrhagic tendency and the diverse clinical effects that arise from deficiencies in various components of the two pathways. Hoffman’s group has thus proposed a cell-based model of hemostasis (33) that emphasizes interactions between clotting factors and specific cell surfaces, and which appears to illuminate many of the unresolved issues associated with the cascade model.

Fig. 3. New concept of hemostatic pathway (cell-based model of hemostasis)
The cell-based model emphasizes that coagulation occurs in a series of three overlapping steps that take place on different cell surfaces, rather than as a cascade that produces an abundance of activated factors and inevitably leads to clot formation. The first phase (INITIATION) occurs on a TF-bearing cell. In the second phase (AMPLIFICATION), platelets and co-factors are activated in order to prepare for large-scale thrombin generation. Finally, the third phase (PROPAGATION) occurs on the surface of platelets, and results in the production of large amounts of thrombin. (modified from Hoffman et al., Ref. 31).

Hemostatic factors and cardiac events

Epidemiological studies have revealed a positive correlation between the risk of acute cardiovascular events and plasma coagulation factor levels, such as fibrinogen and factor VII (5, 34). Moreover, basic investigations have identified fibrin/fibrinogen antigens in evolving atherosclerotic plaques, suggesting a smooth muscle mitogenic effect of the products of fibrinogen to fibrin degradation (35). Correlations between decreased fibrinolytic potential and increased risk are similar (36), and are due to either increased levels of plasminogen activator inhibitor type I or perhaps to plasminogen inhibition due to high levels of circulating Lp (a) (37).

Since the key factor precipitating an acute ischemic event is plaque disruption, systemic levels of fibrinolytic or thrombotic activity at that moment probably determine the magnitude of thrombosis. Levels of fibrinopeptide A are increased in patients with unstable angina and acute myocardial infarction (38). These levels fall within a few days of the acute event and provide yet more evidence of the thrombotic origin of acute myocardial infarction. Plasma levels of prothrombin activation fragments F1 and F2 that indicate thrombin generation can persist for up to 6 months after an acute event (39). Further efforts to define the role of the hemostatic system in
Atherosclerosis are important because pharmacological manipulation of hemostatic risk factors, such as agents that lower fibrinogen levels should confer a therapeutic benefit.

The expression of TF on the surface of monocytes readily induces their procoagulant activities. When exposed, abundant TF within plaque is the major factor responsible for the thrombogenic potential of the lipid core (40). The expression of TF by circulating monocytes increases during acute ischemic events (41). The proportion of monocytes expressing TF is increased in patients with acute myocardial infarction and unstable angina compared with normal individuals and those with stable angina (41). The evidence suggests that monocyte activation in the peripheral blood is associated with the onset of acute coronary syndromes (42, 43).

**Hemostatic factors and atherogenesis**

Evidence that the intrinsic pathway is involved in atherothrombosis has been generated from several clinical studies and epidemiological surveys. These include investigations of patients with hemophilia A who have bleeding disorders due to a genetic deficiency of FVIII, a key component of the intrinsic pathway. Long-term clinical observations have revealed that mortality due to myocardial infarction is reduced 5-fold among such patients (44, 45). Although they have fewer atherosclerotic plaques (8), atherosclerotic risk factors between hemophilic patients and normal individuals do not significantly differ (46). Therefore, the lower mortality in hemophiliacs is considered to be associated with impaired coagulation (46). The plasma level of FVIII in increased in patients with myocardial infarction. Although whether FVIII elevation is a cause or a consequence of disease progression remains debatable, an elevated concentration of FVIII is an accepted risk factor for atherothrombosis. Clinical observations indicate that both intrinsic and extrinsic coagulation pathways are involved in atherothrombosis, but the biochemical mechanisms responsible for the activation of these pathways at lesion site have not been completely elucidated.

The deposition of small mural thrombi onto the intima following endothelial denudation is considered to be a major mechanism of plaque growth. A high proportion of plaques consists of connective tissue synthesized by SMCs. Many animal studies have shown that focal mechanical denudation of the endothelium produces small thrombi (49, 50). This is followed by intimal smooth muscle proliferation at the injured site, and leads to raised white fibrous plaque. Residual thrombus is often identified in the plaques, as observed in autopsies (Fig. 2).

**Tissue factor/factor VIIa/protease activated receptor 2**

Thrombus formation is a key event in the development of intimal thickening, which is considered to comprise the early stage of atherosclerotic plaque formation. Many studies, including ours (10, 11, 13, 51, 52, and reviewed in 53) have demonstrated that TF in atherosclerotic lesions contributes to atherogenesis. Although native TF itself has no intrinsic protease activity, the bimolecular complex of TF and FVIIa results in enhancement of the FVIIa catalytic domain and activates FIX and FX, which leads to thrombin generation. PARs comprise a family of seven-transmembrane G-protein-coupled receptors. Serine protease cleavage at the N-terminus activates PARs, which then generates a new tethered ligand that interacts with the receptors within extracellular loop-2. The following PAR receptors have been identified to date: PAR1, PAR2, PAR3 and PAR4. Thrombin is a powerful activator of PAR1, PAR3 and PAR4, but other proteases can also cleave these receptors and thus might physiologically contribute to their function (54–56). Several trypsin-like serine proteases, including trypsin, trypase, and FVIIa and Xa, activate PAR2, but thrombin does not (57). These PAR-activating proteases, especially the coagulants, mediate the responses that are critical for hemostasis and thrombosis, as well as inflammatory and proliferative reactions triggered by tissue damage (54, 58, 59). Although PARs might play important roles in normal or pathological states, which protease(s) and PAR(s) function in specific cellular processes remain unclear. Table 1 shows the roles of PAR2 in the cardiovascular system. Thrombin signaling, mediated by PARs 1, 3 and 4 contributes to atherogenesis [reviewed in (60)], but the participation of PAR2 in atherosclerotic lesions has not been elucidated. Some reports have demonstrated that PAR2 is expressed in aortic walls and increases after balloon injury (61, 62). We identified PAR2 immunoreactivity in the intima and media of coronary atherosclerotic lesions and also in cultured aortic SMCs (63). We also reported that the PAR2-activating peptides of exposed tethered PAR2 ligand, induce SMC migration, which is comparable to that induced by TF/FVIIa complex and platelet-derived growth factor-BB. The contribution of PAR2 to inflammatory responses has been evaluated in PAR2-deficient mice (64–67). Further examination, especially of the cardiovascular system including atherogenesis, should confirm the role of PAR2 in atherosclerosis.
Conclusion

Hemostatic factors play a pivotal role in the development and complications of atherosclerosis through thrombogenic activities and other multifunctional properties. Understanding how hemostatic factors are modulated should lead to the development of novel therapeutic strategies against atherogenesis and acute coronary events.

Acknowledgment: This work was partly supported by Grants-in-Aid for the 21st Century COE program (Life Science) and for Scientific Research (No.16590321) from The Ministry of Education, Science, Sports and Culture, Japan.

References


Table 1. PAR-2 activation in cardiovascular system.

<table>
<thead>
<tr>
<th>In vivo</th>
<th>Vasodilation</th>
<th>Mouse</th>
<th>Damiano et al. (68), 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vasodilation</td>
<td>Rat</td>
<td>Hwa et al. (69), 1996</td>
</tr>
<tr>
<td></td>
<td>Angiogenesis</td>
<td>Mouse</td>
<td>Milla et al. (70), 2002</td>
</tr>
<tr>
<td>Ex vivo</td>
<td>Vasorelaxation</td>
<td>Rat</td>
<td>Al-Ani et al. (71), 1995</td>
</tr>
<tr>
<td></td>
<td>Smooth muscle contraction</td>
<td>Rabbit</td>
<td>Komuro et al. (72), 1997</td>
</tr>
<tr>
<td></td>
<td>Vascular contraction</td>
<td>Rat</td>
<td>Roy et al. (73), 1998</td>
</tr>
<tr>
<td>In vitro</td>
<td>EC proliferation</td>
<td>Human</td>
<td>Mirza et al. (74), 1996</td>
</tr>
<tr>
<td></td>
<td>SMC proliferation</td>
<td>Human</td>
<td>Bono et al. (75), 1997</td>
</tr>
<tr>
<td></td>
<td>SMC Proliferation</td>
<td>Bovine</td>
<td>Bretschneider et al. (76), 1999</td>
</tr>
<tr>
<td></td>
<td>SMC migration</td>
<td>Human</td>
<td>Marutsuka et al. (64), 2002</td>
</tr>
<tr>
<td></td>
<td>PGI2 formation</td>
<td>Human</td>
<td>Molino et al. (77), 1997</td>
</tr>
<tr>
<td></td>
<td>TF formation</td>
<td>Human</td>
<td>Aim et al. (78), 1999</td>
</tr>
<tr>
<td></td>
<td>vWF release</td>
<td>Human</td>
<td>Storck et al. (79), 1996</td>
</tr>
<tr>
<td></td>
<td>IL-6 and IL-8</td>
<td>Human</td>
<td>Shpacovitch et al. (80), 2002</td>
</tr>
<tr>
<td></td>
<td>Cardiomyocyte signaling</td>
<td>Rat</td>
<td>Sabri et al. (81), 2000</td>
</tr>
</tbody>
</table>


(20) Tanaka K and Sueishi K: The coagulation and fibrinolytic systems and atherosclerosis. Laboratory Invest, 69: 5–18, 1993


(39) Merlini P, Bauer K, Oltrona L, Ardissino D, Cattaneo M, Belli C, Manucci PM, and Rosenberg RD: Persistent activation of coagulation mechanism in un-


(68) Diamino BP, Cheung WM, Santulli RJ, FungLeung WP, Ngo K, Ye RD, Darrow AL, Derian CK, DeGara-villa L, and Andrade-Gordon P: Cardiovascular response mediated by protease-activated receptor-2 (PAR-2) and thrombin receptor (PAR-1) are distinguished in mice deficient in PAR-2 or PAR-1. J Pharmacol Exp Ther, 288: 671–678, 1999


