Blood Coagulation and Atherothrombosis

Hiroshi Ashikaga
Kenneth R. Chien

Atherothrombosis, or thrombosis at atherosclerotic sites, is the main culprit in the pathogenesis of myocardial infarction and stroke. Therefore, it is also the major factor responsible for atherosclerosis-related morbidity and mortality. Coagulation pathways play a crucial role in the pathogenesis of atherothrombosis by facilitating the generation of occlusive thrombotic plugs at sites of ruptured atherosclerotic plaques. Although the major contribution of the extrinsic pathway of coagulation to the initiation of atherothrombosis is well established, recent observations have indicated involvement of the intrinsic coagulation pathway as well. However, the understanding of biochemical mechanisms involved in activation of these pathways at atherosclerotic lesions is still incomplete.

NORMAL COAGULATION PATHWAYS

Coagulation pathways are dependent on a group of proteins termed coagulation factors that are normally present in inactive proenzyme forms (Table 28-1). The sequential activation of coagulation factors forms a coagulation cascade that eventually results in fibrin clot formation. The blood coagulation system includes two pathways composed of distinct groups of coagulation factors: the extrinsic or tissue factor-dependent pathway and the intrinsic pathway. The final step in both the extrinsic and the intrinsic pathways is the activation of factor X (fX) to factor Xa (fXa). Generation of fXa merges both pathways in a common pathway that leads to the production of the multifunctional molecule thrombin (Fig. 28-1). In addition, several mechanisms to counteract the coagulation cascade exist to maintain intact blood circulation and prevent clotting. These mechanisms, including antithrombin, the protein C/protein S/thrombomodulin system, and tissue factor pathway inhibitor (TFPI), regulate the coagulation cascade at different levels to mitigate clot formation under physiologic circumstances (Fig. 28-2).

Activation of the extrinsic pathway begins with binding of tissue factor (TF), a cell-surface glycoprotein, to an activated serine protease fVIIa. Exposure of TF to circulating blood is caused by disruption of the endothelial layer as in vascular injury or by heterotrophic TF expression in different cell types in response to various stimuli. Small amounts of fVIIa are present (1% to 2%) and circulate in the blood. TF on the cell surface binds to free fVIIa in the plasma to form the TF/fVIIa complex. Both proteins possess low enzymatic activity in their free forms, but the TF/fVIIa complex acts as a potent enzyme to further activate free fVII to generate fVIIa, producing more TF/fVIIa complexes to amplify the initial trigger (TF-mediated fVII autoactivation). The TF/fVIIa complex then activates IX to yield fIXa, either directly or indirectly by initially converting fIX to fIXa, which subsequently activates IX in the presence of fVIIIa.

The intrinsic pathway is triggered by the autoactivation of fXII to fXIIa, which subsequently initiates the cascade of sequential activation of fXI and fIX to generate fXa. Then fXa catalyzes the conversion of fIX to fIXa, but this reaction requires an activated form of another coagulation factor fVIIIa, which is generated by thrombin-mediated activation of fVIII.

Factor Xa, the end product of both the extrinsic and intrinsic pathways, triggers the common pathway of coagulation by converting prothrombin to thrombin, which in turn initiates formation of fibrin from fibrinogen. The conversion of prothrombin to thrombin requires a cofactor fVa, which is produced by thrombin-mediated activation of fV. Thrombin also activates fXIII to form fXIIIa, which catalyzes the formation of cross-linked fibrin polymer.

Antithrombin (antithrombin III) is a plasma protease inhibitor that inactivates thrombin and other activated coagulation factors in the intrinsic and common pathways by binding to the active site of these enzymes. The anticoagulant heparin's major mechanism of action is to accelerate the formation of these neutralizing complexes.

Protein C is a plasma glycoprotein that is activated by thrombin, and activated protein C (APC) is a potent anticoagulant that inactivates fVa and fVIIIa through the thrombin-thrombomodulin complex. The thrombin-induced activation of protein C occurs physiologically on thrombomodulin, a transmembrane proteoglycan-binding site for thrombin on endothelial cell surfaces. The rate of this reaction is increased by a cofactor, protein S, which increases the affinity of APC for phospholipids in the formation of the membrane-bound protein C complex (Fig. 28-3).

TFPI is the endogenous inhibitor of the TF/fVIIa complex (Fig. 28-4). TFPI is a multivalent Kunitz-type serine protease inhibitor, consisting of three tandem Kunitz domains, which exerts inhibitory effects against the TF/fVIIa complex and fXa, thereby regulating the extrinsic pathway of coagulation. Endothelial cells are the principal source of plasma TFPI.

(continued)
It has recently been recognized that the coagulation mechanism involves the assembly of multiprotein complexes on the phospholipid cellular membrane, including the extrinsic and intrinsic Xase (tenase) complex, prothrombinase complex, and protein Cae complex (Fig. 28-3, Table 28-2). Each complex consists of an enzyme, its zymogen substrate, and its cofactor on the phospholipid membrane surface. The formation of these complexes on the cell membrane surface promotes the reactions in the coagulation and anticoagulation pathways.

**TABLE 28-1  BLOOD CLOTTING FACTORS AND INHIBITORS**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Chromosome</th>
<th>Gene (kb)</th>
<th>mRNA (kb)</th>
<th>Plasma (Half-Life)</th>
<th>Concentration (nm)</th>
<th>Clinical Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin</td>
<td>11</td>
<td>21</td>
<td>2</td>
<td>2.5</td>
<td>1400</td>
<td>H –</td>
</tr>
<tr>
<td>Factor V</td>
<td>1q 21–25</td>
<td>80</td>
<td>7</td>
<td>0.5</td>
<td>20</td>
<td>H + +</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Xq 28</td>
<td>186</td>
<td>9</td>
<td>0.3–0.5</td>
<td>0.7</td>
<td>H + –</td>
</tr>
<tr>
<td>Factor VII</td>
<td>13</td>
<td>12.8</td>
<td>2.5</td>
<td>0.25</td>
<td>10</td>
<td>H + –</td>
</tr>
<tr>
<td>Factor IX</td>
<td>X</td>
<td>34</td>
<td>2.8</td>
<td>1</td>
<td>90</td>
<td>+</td>
</tr>
<tr>
<td>Factor X</td>
<td>13q 32-pter</td>
<td>27</td>
<td>1.5</td>
<td>1.25</td>
<td>170</td>
<td>+</td>
</tr>
<tr>
<td>Factor XI</td>
<td>4q 35</td>
<td>23</td>
<td>2.1</td>
<td>2.5–3.3</td>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>Factor XII</td>
<td>5q 35-pter</td>
<td>12</td>
<td>2.6</td>
<td>2–3</td>
<td>375</td>
<td>–</td>
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<tr>
<td>High molecular</td>
<td>5q 26-pter</td>
<td>27</td>
<td>2.015</td>
<td>5</td>
<td>600</td>
<td>–</td>
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<td>Prekallikrein</td>
<td>4q34-q35</td>
<td>22</td>
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<tr>
<td>Factor XIIa</td>
<td>6 p 24–25</td>
<td>&gt;160</td>
<td>5.8</td>
<td>9–10</td>
<td>70</td>
<td>+ –</td>
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<tr>
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<td>1 q31-q32 1</td>
<td>28</td>
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<td></td>
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<td>Tissue factor</td>
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<td>12.4</td>
<td>2.3</td>
<td>–</td>
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<td></td>
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<td>Protein C</td>
<td>2q 14–21</td>
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<td>1.7</td>
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<tr>
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<td>5.5</td>
<td>1.75</td>
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</tr>
<tr>
<td>Thrombomodulin</td>
<td>20p 12-cen</td>
<td>5.7</td>
<td>5.7</td>
<td>–</td>
<td></td>
<td></td>
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<tr>
<td>Fibrinogen</td>
<td>4q 25q32</td>
<td>50</td>
<td>3–5</td>
<td>8800</td>
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<td>α chain</td>
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<td>5.4</td>
<td>2.2</td>
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<td></td>
</tr>
<tr>
<td>β chain</td>
<td>8</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ chain</td>
<td>8.5</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Antithrombin III</td>
<td>1 q 22–25</td>
<td>14</td>
<td>1.4</td>
<td>2.5–4</td>
<td>2500</td>
<td>+ –</td>
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<tr>
<td>Heparin cofactor II</td>
<td>22q 11</td>
<td>16</td>
<td>2.3</td>
<td>2.5</td>
<td>1200</td>
<td>+ –</td>
</tr>
<tr>
<td>Tissue factor</td>
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<td>1.4, 4</td>
<td>NA</td>
<td>2.5</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombin activated</td>
<td>13</td>
<td>1.8</td>
<td></td>
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<td>75</td>
<td></td>
</tr>
<tr>
<td>fibrinolysis inhibition</td>
<td></td>
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<td></td>
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</tbody>
</table>

H, Hemorrhagic disease/hemophilia; NA, not available; T, thrombotic disease/thrombophilia.

It has recently been recognized that the coagulation mechanism involves the assembly of multiprotein complexes on the phospholipid cellular membrane, including the extrinsic and intrinsic Xase (tenase) complex, prothrombinase complex, and protein Case complex (Fig. 28-3, Table 28-2). Each complex consists of an enzyme, its zymogen substrate, and its cofactor on the phospholipid membrane surface. The formation of these complexes on the cell membrane surface promotes the reactions in the coagulation and anticoagulation pathways.

**COAGULATION PATHWAYS IN ATHEROSCLEROSIS**

**Extrinsic Pathway**

**Tissue Factor**

The initiation of the coagulation cascade in the pathogenesis of the atherothrombosis has largely been ascribed to the extrinsic, TF-dependent pathway. TF is a 263 amino acid, membrane-bound glycoprotein consisting of three domains: the extracellular (residues 1 to 219), transmembrane (residues 220 to 242), and cytoplasmic (residues 243 to 263) domains. The extracellular domain is responsible for the binding to the IVIIIa, thereby initiating the extrinsic pathway of the coagulation cascade. Normal arteries and veins lack TF mRNA and protein, except for a small amount present in vascular smooth muscle cells (SMECs) in the tunica media and fibroblasts in the adventitia. Under normal conditions, TF is not expressed in peripheral blood cells or endothelial cells. TF is, therefore, physically separated from the bloodstream. In contrast, extensive evidence demonstrates abundant TF expression in all stages of human atherosclerotic lesions. Both TF content and activity are significantly higher in plaques from patients with acute coronary syndrome compared with those with stable angina. Abnormal TF expression and activity are also implicated in postischemic reperfusion injury.

The concept that the extrinsic pathway plays a major role in the initiation of atherothrombosis is strongly supported by the finding that disruption of human atherosclerotic plaques containing high levels of TF results in platelet deposition, which positively correlates with the TF content. In patients with acute coronary syndrome, thrombosis is seen only in TF-positive atherosclerotic plaques, and fibrin deposition occurs mainly around macrophages expressing TF. Inhibition of TF activity in atherosclerotic plaques with recombinant TFPI (rTFPI), antihuman TF antibodies, or adenoviral gene transfer of human TFPI significantly reduces plaque thrombogenicity, inhibiting both platelet and fibrinogen deposition without affecting systemic coagulation status.

The source of this heterotropically expressed TF in atherosclerotic lesions is a number of different cell types that occupy approximately 60% of the cell population in the atherosclerotic plaque: endothelial cells, SMECs, monocytes, macrophages, foam cells, and mesenchymal-appearing intimal cells. Heterotropic TF is
also found in the extracellular matrix surrounding TF-producing foam cells, macrophages, and SMCs, adjacent to the cholesterol clefts and within the necrotic cores of the plaques. Significant platelet deposition is found on the lipid-rich atheromatous core, which also exhibits the most intense TF staining compared with other arterial components. In addition to a membrane-bound form, significantly high levels of circulating soluble form of TF are found in patients with acute coronary syndrome. The soluble TF levels in the blood are an important predictor of clinical outcome that reflects the hypercoagulable state of these patients, and the levels return to normal values after successful treatment with anticoagulation or antiplatelet agents. The blood-borne TF may trigger thrombus formation independent of procoagulants in atherosclerotic plaques, contributing to propagation of thrombus formation at the site of vascular injury. However, the source of the circulating TF is not clear; it may originate from membrane-bound TF at the site of atherosclerosis or from circulating neutrophils and monocytes. It was also discovered that TF is transferred from monocytes and polymorphonuclear leukocytes to platelets, through the interaction of CD15 and TF with platelets. This transfer phenomenon enables platelets to initiate and propagate thrombus formation independent of procoagulants in atherosclerotic plaques, contributing to propagation of thrombus formation at the site of vascular injury.

Factor VII

The fVII gene (12.8 kb) is encoded on chromosome 13 and yields mRNA of 2.5 kb. Factor VII (50 kd) is a vitamin K-dependent coagulation factor that circulates in plasma predominantly as a single-chain inactive zymogen. It is activated by fIXa, fXla, fXa, fVIIa, and thrombin through a single peptide bond cleavage at Arg152-Ile153. The activated form of fVII, fVIIa, contains a light chain (20 kd) and a heavy chain (30 kd) that are covalently linked by a disulfide bond. High levels of fVII in the plasma are associated with increased risk of ischemic events, whereas low plasma
fVII levels appear to attenuate the risk. In a study of an arginine-glutamine mutation at amino acid 353 (R353Q) and an H7H7 polymorphism in the hypervariable region 4 of intron 7 of fVII, patients with the QQ or H7H7 genotype had significantly lower levels of both fVII antigen and clotting activity and a lower risk of myocardial infarction than those with the RR or H6H6 genotype. Another polymorphism in intron 1a of the fVII gene was identified; it is caused by the nucleotide change G to A at position +73 (G73A), which may be in a strong linkage disequilibrium with the Q353 allele. Patients with both 73A and Q353 alleles had lower fVII levels and lower risk of myocardial infarction than individuals without the mutation. These findings suggest that polymorphisms of the fVII gene may attenuate the risk of atherothrombosis, presumably through alterations in the plasma fVII levels.

Intrinsic Pathway

**Factor XII**

The fXII gene, consisting of 14 exons and 13 introns on chromosome 5, gives rise to an mRNA of 2.4 kb. The mature fXII protein is composed of 596 amino acid residues (80 kd) in a single polypeptide chain. Factor XII, or Hageman factor, is the first coagulation factor of the intrinsic pathway and is activated by plasma kallikrein to form fXIIa. This activation process is facilitated by high molecular weight kininogen (HMWK) and by contact with negatively charged surfaces such as glass or collagen. Factor XIIa converts fXI to fXI and prekallikrein to kallikrein. However, patients with fXII deficiency do not develop a bleeding diathesis, and fXIIa does not appear to play a significant role in the coagulation cascade in vivo.

**Factor XI**

The fXI gene (23 kb) is located on chromosome 4 and contains 15 exons and 14 introns. The fXI protein (160 kd) consists of two identical polypeptide chains linked by a disulfide bond. These identical polypeptide chains are cleaved by fXIIa at Arg369-Ile370 in the presence of HMWK and α-thrombin to yield active sites in each of the polypeptide chains. A deficiency of fXI is associated with bleeding, whereas high levels of plasma fXI are a risk factor for venous thromboembolism. No polymorphism of fXI that is associated with increased or decreased incidence of arterial thrombosis has been reported, and the role of fXI in the pathogenesis of acute coronary syndrome remains unclear.
Factor IX

Factor IX is one of the critical components of the intrinsic pathway, and its deficiency leads to hemophilia B. The fIX gene (34 kb), located on X chromosome, gives rise to an mRNA of 2.8 kb. The mature protein (56 kd) requires vitamin K for its synthesis. Factor IX can be activated by the TF/fVIIa complex in the extrinsic pathway or fXIa in the intrinsic pathway. Conversion of fIX to fIXa results in the expression of the fVIII-binding site and the active enzyme site with maximal procoagulant activity. The significance of alterations in fIX in arterial and venous thrombosis has not been established.

Factor VIII

Factor VIII (187 kb) is encoded on the X chromosome, and is divided into 26 exons. It is synthesized as a single-chain protein consisting of more than 2200 amino acids, which undergoes post-translational modifications. The mature, circulating form of fVIII is a heterodimer (330 kd) consisting of a heavy chain and light chain. Thrombin cleaves at least four peptide bonds in fVIII, and the cleavage of the peptide bonds at Arg 372-373 and Arg 1686-1689 are required for fVIII activation. The activation process generates fragments A1 and A2 from the heavy chain and A3-C1-C2 from the light chain, resulting in the heterotrimer fVIIIa molecule. Functional or absolute fVIII deficiency results in hemophilia A, the most common hereditary bleeding disorder.

A number of epidemiologic observations in hemophilia patients, who have a bleeding tendency because of perturbation of the intrinsic pathway, support the involvement of the intrinsic pathway in atherothrombosis. In a cohort study of 919 males with either hemophilia A or B over a period of 20 years, a fivefold reduction in mortality from myocardial infarction was reported compared with that of the general male population. The risk ratio based on the atherosclerosis risk factor profile of hemophilia patients compared with healthy controls was 0.78, which accounts for only a part of the significant risk reduction of coronary artery disease-related mortality. Although these epidemiologic studies strongly support the protective effects of hemophilia on ischemic heart disease, the evidence for protective effects on atherogenesis is conflicting. However, high plasma fVIII levels are an important predictor of unfavorable outcome in patients with acute coronary syndrome, and they correlate with coronary artery disease, carotid atherosclerosis, and venous thromboembolism. Elevated fVIII may well be a consequence of active thrombin formation, and whether fVIII participates in atherogenesis independent of the extrinsic pathway is still uncertain.

Common Pathway

Factor X

Factor X, one of the vitamin K-dependent serine proteases, plays a crucial role in the coagulation cascade as the first enzyme in the common pathway of thrombus formation. The fX gene (27 kb), located on chromosome 13, is composed of eight exons, each of which encodes a specific functional domain within the protein. The mature protein (59 kDa) is comprised of a covalently linked heavy chain (42 kd) and a light chain (16.5 kd). The cleavage of the heavy chain at Arg 194-195 yields its active form fXa. Factor X is at the confluence of the extrinsic pathway and the intrinsic pathway and can be activated by the TF/fVIIa complex or the fIXa/fVIIIa complex.

A deficiency of fX, which is one of the rarest of the hereditary bleeding disorders, exhibits an autosomal recessive inheritance. Homozygous fX deficiency has an incidence of 1:1,000,000 in the general population. Heterozygotes are often clinically asymptomatic. A deficiency of fX may also be acquired, usually in association with amyloidosis. A number of novel anticoagulant strategies to target fXa have been investigated, and some of them have been proven effective in preventing thrombosis in clinical settings (see the section on anticoagulation therapies).

Factor V

The fV gene (80 kb), located on chromosome 1, encodes a 6.8-kb mRNA. Factor V is a glycoprotein that circulates in blood in the plasma and in platelets.

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**TABLE 28-2 COAGULATION FACTOR ENZYME COMPLEXES**

<table>
<thead>
<tr>
<th>Enzyme Substrate</th>
<th>Cofactor</th>
<th>End product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrinsic Xase complex</td>
<td>TF/fVIIa complex</td>
<td>fIX and fX</td>
</tr>
<tr>
<td>Intrinsc Xase complex</td>
<td>fIXa</td>
<td>fIX</td>
</tr>
<tr>
<td>Prothrombinase complex</td>
<td>fIXa</td>
<td>prothrombin</td>
</tr>
<tr>
<td>Protein Case complex</td>
<td>thrombin</td>
<td>protein C</td>
</tr>
</tbody>
</table>
The activated form of $\mathrm{F}V$, or $\mathrm{FV}a$, is a critical component of the coagulation cascade; it acts as a cofactor for $\mathrm{FX}a$ in the prothrombinase complex to convert prothrombin to thrombin and as a cofactor for $\mathrm{APC}$ to degrade $\mathrm{fVIIa}$.

The factor V Leiden, first described in 1993\(^{70}\) and present in approximately 5% of Caucasians, is the most studied mutation in $\mathrm{FV}$ and is related to an increased risk of thrombosis. It is the result of a point mutation in the $\mathrm{FV}$ gene on chromosome 1 (1691GA), resulting in an arginine-glutamine substitution at amino acid 506 (R506Q), the site where APC cleaves $\mathrm{FVa}$.\(^{71}\) This mutation confers APC resistance on $\mathrm{FVa}$, resulting in continued activation of thrombin in the prothrombinase complex. The factor V Leiden mutation is seen in up to 50% of patients with venous thromboembolism,\(^{71}\) and heterozygotes and homozygotes have approximately an 8-fold and an 80-fold increased risk for venous thrombosis, respectively.\(^{72}\) However, the association between factor V Leiden and arterial thrombosis is not clear.

**Prothrombin**

The prothrombin gene (21 kb) is encoded on chromosome 11 and consists of 14 exons.\(^{73,74}\) The $\mathrm{FXa}/\mathrm{FVa}$ complex formed on membranes in the presence of $\mathrm{Ca}^{2+}$ is known as the prothrombinase complex, which facilitates conversion of prothrombin to thrombin.

Prothrombin deficiency is a rare form of coagulation disease with hemorrhagic manifestations that is inherited in an autosomal recessive manner. Conversely, a common mutation of the prothrombin gene is associated with elevated prothrombin levels in blood and an increased risk of both arterial and venous thrombosis. A G-to-A transition at position 20210 (20210GA) in the sequence of the 3′-UT region of the prothrombin gene was identified, which is present in 1% to 2% of the general population. The 20210GA mutation is associated with elevated plasma levels of prothrombin, thrombin-antithrombin complex, and prothrombin fragment 1+2. Patients with the prothrombin 20210GA mutation are at an increased risk of myocardial infarction, ischemic stroke,\(^{76,77}\) and venous thromboembolism.\(^{78}\) The thrombophilic tendency of the 20210GA mutation is also reflected by a significantly increased endogenous thrombin potential (ETP) found in heterozygotes of the 20210GA mutation; homozygotes have an even higher ETP compared with controls.\(^{79}\)

**Fibrinogen**

Fibrinogen is encoded by three separate genes, and each gene encodes one of the three subunits of fibrinogen (\(\alpha\), \(\beta\), and \(\gamma\)). These three genes are closely linked in a 50-kb region of chromosome 4: the \(\alpha\) gene in the middle flanked by the \(\beta\)-gene on one side and the \(\gamma\)-gene on the other.\(^{80}\) The regulation of these genes is coordinated at the transcriptional level to yield simultaneous production of the three fibrinogen polypeptide chains.\(^{81}\) The \(\alpha\), \(\beta\), and \(\gamma\) chains are highly homologous in their amino acid sequence, which suggests that they evolved from a common ancestral gene.\(^{82-85}\) The mature form of fibrinogen is a glycoprotein (340 kDa) that consists of three pairs of polypeptide chains: two \(\alpha\)\(\alpha\) chains, two \(\beta\)\(\beta\) chains and two \(\gamma\)\(\gamma\) chains.\(^{86}\) Fibrinogen circulates in blood as a biologically inactive form. Thrombin converts fibrinogen to its active form, fibrin, by cleaving both \(\alpha\)\(\alpha\) and \(\beta\)\(\beta\) chains. The fibrin monomers noncovalently homopolymerize to form two-stranded prototypifibrils.

High levels of plasma fibrinogen are an independent predictor of cardiovascular disease and are strongly associated with arterial thrombosis including myocardial infarction and stroke.\(^{85,87}\) A large number of mutations in the fibrinogen gene have been described that lead to variable bleeding and thrombotic tendencies. Fibrinogen New York I has a short thrombin time, fast polymerization, and increased platelet aggregation. Fibrinogen Paris V has a single base change that results in the substitution R554C in the \(\alpha\) chain, conferring resistance against plasmin degradation; patients who are heterozygous for this substitution suffer from recurrent thrombotic disorders.\(^{89}\) The \(\alpha\) fibrinogen T312A polymorphism is associated with an increased poststroke mortality in patients with atrial fibrillation.\(^{90}\) Another polymorphism at nucleotide 148 in the \(\beta\) fibrinogen promoter region is associated with an increased risk of carotid atherosclerosis despite normal fibrinogen levels in the plasma.\(^{91}\)

**Factor XIII**

Factor XIII is a zymogen of a cysteine transglutaminase, consisting of two peptide subunits, the \(\alpha\) chain (75 kDa) and the \(\beta\) chain (80 kDa). The \(\alpha\) gene and \(\beta\) gene are encoded on chromosome 6 and 1, respectively.\(^{92,93}\) Factor XIII (320 kDa) is a tetramer of two \(\beta\) chains and two \(\beta\) chains. Thrombin activates FXIII into FXIIa by cleaving the \(\alpha\) chain at Arg37-38. The two-stranded prototypifibrils consist of noncovalently linked fibrin monomers; therefore, the strands are unstable. Activated factor XIII (FXIIa), a transglutaminase, catalyzes the condensation of lysine residues on one chain and glutamic acid residue on the second chain, giving rise to a stable, cross-linked fibrin polymer.\(^{94}\)

Several point mutations in the \(\alpha\) gene result in FXIII deficiency, which presents as a rare autosomal recessive bleeding disorder. A C-to-T transition at Arg661 in exon 14 creates a premature stop codon, resulting in a 10- to 30-fold reduction in FXIII mRNA levels. Another mutation, the T-to-C transition in exon 6, results in a substitution of threonine242 for methionine, but this does not appear to affect the level of mRNA. Both mutations lead to an absence of a functional and immunodetectable FXIII protein, presumably because of an altered conformation of the mutant polypeptide, resulting in early degradation of the defective protein.\(^{95}\) Factor XIII-V34L, a common point mutation (G/T) in exon 2 of the \(\alpha\) gene, may protect against myocardial infarction and venous thromboembolism but predispose to intracranial hemorrhage.\(^{96-98}\)
Natural Anticoagulant Mechanisms

Protein C Pathway

The protein C pathway is a highly coordinated anticoagulation mechanism that converts the coagulation signal generated by thrombin into an anticoagulant response through the activation of protein C by the thrombin-thrombomodulin complex (Fig. 28-2). Protein C and thrombin assemble on the three epidermal growth factor (EGF) repeats of thrombomodulin on the endothelial cell, and thrombin rapidly cleaves the Arg169-Leu170 bond in its heavy, chain yielding APC. APC then interacts with protein S to inactivate two critical coagulation cofactors, fVα and fVIIα, thereby blocking further thrombin generation. Protein S appears to alter the cleavage site preferences of APC in fVα, presumably by changing the distance of the active site of APC relative to the membrane surface.

Protein C (34 kDa) is a vitamin K-dependent zymogen comprised of a heavy chain and a light chain linked by a disulfide bond. The protein C gene (12 kb), containing 9 exons, is encoded on chromosome 2, and yields an mRNA of 1.5 kb. Thrombomodulin, a thrombin receptor on the luminal surface of endothelial cells, serves as a critical cofactor for the protein C anticoagulation pathway. The thrombomodulin gene is encoded on chromosome 20. No introns are present within the coding region; therefore, the EGF type B repeats and a membrane-spanning region are not isolated within discrete exons. The overall genetic structure of thrombomodulin exhibits homology to the human LDL receptor. Thrombomodulin gene expression is down-regulated at the transcriptional level by TNF-α through a signaling cascade that involves binding to a member of the Ets nuclear factor family.

Protein S (60 kDa) is another vitamin K dependent plasma protein encoded by the gene (80 kb) on chromosome 3 that contains 15 exons. Endothelial cell protein C receptor (EPCR) is a member of the CD1/major histocompatibility complex superfamily that binds to both inert protein C and APC in a Ca2+-dependent fashion. Both the function and expression of EPCR are attenuated by exposure of endothelium to TNF-α. EPCR is likely to enhance efficient transfer of protein C to thrombomodulin on endothelial cell surfaces.

Patients with congenital deficiencies in the protein C pathway are at increased risk for both arterial and venous thrombosis. Acute inflammatory disease such as sepsis can result in acquired deficiencies in the protein C pathway with resultant increase in the plasma TNF-α levels, whereas administration of APC decreases the circulating levels of TNF-α. Reduction in plasma protein C levels is associated with poor clinical outcomes in patients with septic shock, and treatment with recombinant APC significantly reduces mortality in patients with severe sepsis. Therefore, the protein C pathway appears to modulate inflammatory response as well as coagulation cascades.

Natural phospholipids including phosphatidylethanolamine and cardiolipin appear to stimulate the anticoagulant protein C pathway by increasing the affinity of phospholipid surfaces for the protein C complex to enhance enzymatic degradation of fVα. Antiphospholipid or anticardiolipin antibodies inhibit this enhancement of protein C pathway, resulting in a clinical thrombophilic tendency. Neutral glycolipids, glucosylceramide, lactosylceramide, and globotriaosylceramide can enhance anticoagulant activity of the protein C pathway by mechanisms distinctly different from those of phospholipids alone, and the deficiency of these glycolipids may be a risk factor for venous thrombosis.

The C allele in a C/T dimorphism at nucleotide 1418 in the thrombomodulin gene is associated with premature myocardial infarction. This polymorphism results in an A455V substitution in the sixth EGF-like domain. Because no association between the C/T dimorphism and the plasma levels of thrombomodulin is observed, the C/T dimorphism may mitigate the function of thrombomodulin as a cofactor for APC.

Antithrombin

Antithrombin, or antithrombin III, is an endogenous anticoagulant that inhibits thrombin and fXa to block the coagulation cascade. Antithrombin also inactivates fXa, fXIIa, and fVIIa but to a lesser extent (Fig. 28-2). The antithrombin gene (15 kb), consisting of 7 exons, is located on chromosome 1, and encodes for a 1.8-kb mRNA that yields the single-chain glycoprotein protease inhibitor antithrombin (68 kd). Antithrombin belongs to the serine protease inhibitor (serpin) superfamily and inactivates thrombin and other activated coagulation factors by forming a complex between the active site of the enzyme and the reactive center (Arg393-Ser394) of antithrombin. Antithrombin contains a C-terminal arginine-serine reactive site that interacts with coagulation factors and two positively charged regions that bind to the sulfated polysaccharides, heparin and heparan sulfate.

Coagulation factors slowly interact with antithrombin in the absence of heparin or heparan sulfate, but the addition of these polysaccharides significantly accelerates the rate of reactions by a factor of several thousand to inactivate coagulation factors. Heparin-bound antithrombin undergoes a conformational change in the reactive sites. Arginine reactive centers of antithrombin bind to the enzyme active center serines of thrombin and other serine protease coagulation factors, thereby neutralizing their activities. Heparin then dissociates from these complexes and can be reused to bind to other antithrombin molecules. Heparin, thus, acts as a catalyst in accelerating the neutralization of thrombin and other activated clotting factors by antithrombin.

Tissue Factor Pathway Inhibitor

TFPI, an endogenous inhibitor of TF (Fig. 28-2), is a plasma protein (34 kd) that exhibits an acidic aminoterminal region, three tandem repeated serine protease inhibitor domains homologous in structure to Kunitz
trypsin inhibitor, and a basic carboxyl-terminal sequence (Fig. 28-4).123,124 The first Kunitz domain (Kunitz 1) binds to the TF/fVIIa complex, whereas the Kunitz 2 domain forms a complex with fXa with assistance from other portions of the molecule. The function of the Kunitz 3 domain remains unknown. The TFPI gene is located on chromosome 2 and contains nine exons and eight introns.

In all types of atherosclerotic lesions of coronary arteries (type I, II, III, and IV), TFPI is increased in the endothelial cells and macrophages125 and appears to attenuate the TF activity in atherosclerotic plaques.126 This may reflect a physiologic response to counteract thrombogenic tendency in atherothrombosis.

Plasma levels of total and free TFPI, possibly originating from endothelial cells and monocytes, are also increased in patients with acute coronary syndrome.127 and a positive correlation is observed between TF and TFPI plasma levels.128 The plasma TFPI levels are higher in patients with unstable angina than those with stable angina and myocardial infarction, and high TFPI levels are associated with unfavorable clinical outcomes.37 Intravenous unfractionated heparin (UFH) administration does not affect TFPI levels but markedly lowers the TF levels, which supports the clinical efficacy of anticoagulation therapy in patients with acute coronary syndrome.129 TFPI is also implicated in a rebound increase in thrombophilic potential after abrupt cessation of intravenous UFH among patients with non-ST segment elevation acute coronary syndrome. After UFH cessation, the plasma level of TFPI decreases while thrombin generation progressively increases. This heparin rebound phenomenon can be attenuated by an abbreviated intravenous heparin (UFH) administration in patients with acute myocardial infarction significantly decreases plasma TFPI levels and surface-associated TFPI on circulating monocytes. This reduction in TFPI level, presumably resulting from enzymatic inactivation of TFPI by plasmin, may contribute to thrombotic complications after fibrinolytic therapy.130

Some patients heterozygous for V264M polymorphism in exon 9 of the TFPI gene exhibit significantly lower plasma TFPI levels than those with the most common genotype. However, no increased incidence of acute coronary syndromes is observed among these heterozygotes.131

**PROCOAGULANT FACTORS IN ATHEROTHROMBOSIS**

**Lipoproteins**

LDL, particularly oxidized LDL, contributes to activation of both the extrinsic and intrinsic pathways of coagulation in atherosclerotic lesions, whereas HDL is a potent stimulator of the anticoagulant protein C pathway.132 Oxidized LDL is atherogenic, and it provides an important mechanistic basis that links atherosclerosis with atherothrombosis. Oxidized LDL induces atherosclerosis by stimulating monocyte infiltration and SMC migration and proliferation. Oxidized LDL contributes to atherothrombosis through several mechanisms: induction of plaque erosion by endothelial cell apoptosis, impairment of the anticoagulant balance in endothelium, stimulation of TF production on SMCs and induction of apoptosis in macrophages.133 Oxidized LDL also provides a phospholipid surface to support the assembly of the prothrombinase complex and the extrinsic and intrinsic Xase complex, thereby contributing to thrombin generation.132,134,135 Therefore, oxidized LDL accumulated in atherosclerotic lesions is likely to play a major role in enhancing the procoagulant activity.

LDL increases synthesis and activity of TF in atherosclerotic lesions, but the presence of oxidation appears to be critical. Native LDL increases TF synthesis in human SMCs, while oxidants, some of which exist in atherosclerotic plaques, activate the TF pathway on the cell surface.136 Oxidized LDL induces both TF synthesis and surface TF pathway activity in SMCs, whereas native LDL, which does induce TF mRNA, does not increase TF activity.137–140 Oxidized LDL also upregulates TF expression in endothelial cells and macrophages141–144 and significantly potentiates the procoagulant activity of the extrinsic pathway in these cells.137,144–146

Macrophages and SMCs can promote the intrinsic, fVIIIa/fXa-dependent activation of fX by supporting the assembly of the intrinsic Xase complex, but the rate of fX activation is much lower than that of activated platelets.147 Oxidized LDL enhances the ability of these cells to support the activity of the intrinsic Xase complex and prothrombinase complex, resulting in a significant increase in thrombin formation. The intrinsic pathway may, thus, contribute to the procoagulant activity of atherosclerotic lesions on denudation of the endothelial layer and exposure of macrophages and SMCs to blood. The contribution of the intrinsic, fVIII-dependent pathway is also supported by the finding on human atherectomy specimens that fVIII is present adjacent to macrophages and SMCs in atheromatous areas with large deposits of oxidized LDL.148 In endothelial cells, oxidized LDL does not facilitate the intrinsic pathway, but it increases the thrombogenicity in the extrinsic pathway by stimulating TF expression and by reducing protein C activation.146

Endocytosed oxidized LDL induces severe impairment of lysosomal degradation mechanisms in macrophages not only by partially inactivating lysosomal enzymes but also by destabilizing the acidic vacuolar compartment, leading to relocation of lysosomal enzymes to the cytosol.149 This may result in incomplete scavenger clearance of apoptotic microparticles, which are thrombogenic and contribute to atherothrombosis.

LDL receptor-related protein (LRP) is a multifunctional cell-surface receptor that binds and mediates the endocytosis of several structurally and functionally distinct ligands. LRP is involved in a variety of biologic processes, including the regulation of the coagulation-fibrinolytic balance, lipoprotein metabolism, cellular migration, proliferative processes, and degenerative diseases, thus implicated in the development of atherosclerosis. There is a significant correlation between increased LRP mRNA levels and atherosclerotic plaque progression.150 LRP is
not only diet and exercise but also pharmacologic therapy appear to be effective in improving the obesity-associated procoagulant profile. Thiazolidinediones have an antinflammatory action that may be beneficial for obese nondiabetics as well as diabetics in reducing the risk of atherothrombosis. Administration of troglitazone in obese, nondiabetic subjects results in significant reduction of plasma levels of insulin without significant change in plasma glucose levels. The proinflammatory transcription factor NF-κB in mononuclear cells is downregulated, and the levels of NF-κB-regulated inflammatory mediators—TNF-α, soluble intercellular adhesion molecule (sICAM)-1, monocyte chemoattractant protein (MCP)-1, and PAI-1—significantly decrease. Plasma C reactive protein (CRP) concentration also decreases, whereas the plasma level of IL-10, an anti-inflammatory cytokine, significantly increases. Reduction
in PAI-1 levels in troglitazone-treated obese nondiabetic patients is mainly affected by weight loss.176

Homocysteine

Elevated plasma homocysteine levels are associated with arterial and venous thrombosis as well as atherosclerosis. Inherited forms of hyperhomocysteinemia, including mutations or polymorphisms that lead to functional deficiency of cystathionine-β-synthase, methylenetetrahydrofolate reductase, or methionine synthase, often result in marked elevation of homocysteine in the plasma, whereas acquired forms usually present with intermediate to mild increase in plasma homocysteine levels. These acquired forms of hyperhomocysteinemia include vitamin deficiencies (B12, folate, and B6), renal insufficiency, homocysteinemia due to deficiency of cystathionine-β-synthase, or methylenetetrahydrofolate reductase, or methionine synthase, often result in marked elevation of homocysteine in the plasma, whereas acquired forms usually present with intermediate to mild increase in plasma homocysteine levels. These acquired forms of hyperhomocysteinemia include vitamin deficiencies (B12, folate, and B6), renal insufficiency, hyperhomocysteinemia due to deficiency of cystathionine-β-synthase, or methylenetetrahydrofolate reductase, or methionine synthase, often result in marked elevation of homocysteine in the plasma, whereas acquired forms usually present with intermediate to mild increase in plasma homocysteine levels. These acquired forms of hyperhomocysteinemia include vitamin deficiencies (B12, folate, and B6), renal insufficiency, hypothyroidism, psoriasis, inflammatory bowel disease, and rheumatoid arthritis.177

Homocysteine is a potent procoagulant amino acid that exerts different effects on practically all the pathways involved in the coagulation system. In endothelial cells, homocysteine activates TF and subsequent formation of the intrinsic Xase complex to promote FX activation.178 Homocysteine also irreversibly inhibits thrombomodulin (TM) surface expression on endothelial cells, thus inactivating the protein C pathway.179,180 Homocysteine binds to Vα to confer APC resistance by modification of free cysteine(s) on Vα.181 This functional APC resistance as a result of Vα homocysteinylation may account for the high levels of plasma APC in hyperhomocysteinemic patients with a history of venous thromboembolism.182 Homocysteine enhances both TF expression and activity in endothelial cells and monocytes in a dose-dependent fashion, thereby activating the extrinsic pathway.183,184 Homocysteine reduces the antithrombin protein level on the endothelial cell surface in a dose-dependent fashion, and homocysteine-treated endothelial cells exhibit a substantial reduction in antithrombin binding capacity of heparan sulfate that is mediated by the generation of hydrogen peroxide through alteration of the redox potential.185 Furthermore, homocysteine inhibits the fibrinolytic pathway by directly blocking the t-PA binding domain of annexin II, a phospholipid-binding protein that mediates binding of t-PA to endothelial cells.186 Hyperhomocysteinemia is also a significant predictor of elevated plasma levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, which is associated with atherosclerotic disease and ischemic stroke.187

Angiotensin II

Angiotensin II induces an increase of both synthesis and activity of TF and PAI-1 in endothelial cells, which are effectively inhibited by ACE inhibitors,188 type I angiotensin II receptor (AT1) antagonists,189 natriuretic peptides,190,191 or adrenomedullin.192 The angiotensin II-induced TF gene transcription is mediated by NF-κB, activator protein-1 (AP-1) and at least in part by endothelin receptors (A/B).193,194 The significant role of angiotensin II in activating the TF pathway is supported by a recent discovery that autoantibodies directed at the AT1 receptor found in the serum of preeclamptic patients, whose placentas are often infarcted and express TF, stimulate the AT1 receptor and initiate a signaling cascade resulting in TF expression.195 The angiotensin-II-mediated induction of TF may account for the potency of the inhibition of the renin-angiotensin system in preventing acute coronary syndromes, in addition to its antihypertensive and antihypertrophic effects.

Growth Factors

PDGF-BB stimulates SMCs to produce MCP-1196; both are potent chemotactic and activating factors that can separately induce a dose-dependent TF expression on human SMCs and monocytes.197–199 Active TF on the surface of SMCs induced by PDGF represents only approximately 20% of total TF in the cell, and the remaining TF is present as encrypted surface TF and also in an intracellular pool. This may be an important mechanism to limit the thrombophilic potential of viable SMCs exposed to growth factor stimulation, whereas the encrypted surface TF and intracellular pools may provide a rich source of TF on disruption of cellular architecture of SMC during atherosclerotic plaque rupture or balloon arterial injury.199 TF expression is also induced by VEGF on the surface of endothelial cells in the presence of TNF-α. VEGF and TNF-α synergistically increases TF mRNA, protein, and total activity.200

C Reactive Protein

CRP is an acute-phase reactant, and clinical studies have shown that high levels of CRP positively correlate with poor outcome in patients with acute coronary syndrome.201 CRP promotes atherogenesis through effects on monocytes and endothelial cells, and recent evidence suggests that CRP also contributes to atherothrombosis, especially in diabetics. CRP induces the production of TF in monocytes, increasing thrombogenicity in the extrinsic pathway.202 CRP also induces PAI-1 expression and activity in a time- and dose-dependent fashion in endothelial cells, and the induction is pronounced under hyperglycemic conditions.167 Observational studies have shown a strong association between a family history of type 2 diabetes and high plasma CRP concentrations in nonsmoking healthy adult women.203

CD40/CD40 Ligand

The induction of TF protein and activity is also caused by ligation of CD40 receptor by T cells, activated platelets, or CD40 ligand (CD40L).204,205 CD40L is an immunoregulatory signaling molecule expressed by human vascular endothelial cells, SMCs, human macrophages, and CD4+ T cells. CD40L and its receptor CD40 are coexpressed on these cell types in human atherosclerotic lesions.206 Stimulation of human monocytes, macrophages, or SMCs through CD40 by CD40L induces active TF expression on these cells.207–209 Recent clinical studies have underscored the major role of CD40L in atherothrombosis. Patients with unstable
coronary artery disease who have elevated serum levels of soluble CD40L are at an increased risk of cardiovascular events, and the risk is significantly reduced by antiplatelet treatment with abciximab.210

Cytokines

A number of inflammatory cytokines present in atherosclerotic lesions are found to induce TF expression, thereby contributing to thrombophilia in the extrinsic pathway of coagulation. Th1-derived cytokines, including IFN-γ induce TF production in human monocytes in the presence of activated T cells, whereas Th2-derived cytokines (IL-4, IL-13, and IL-10) inhibit TF production.211,212 In human endothelial cells, TF expression is also induced by IL-12 and TNF-α.214–216

ANTICOAGULATION THERAPIES

Conventional Agents

Unfractionated/Low Molecular Weight Heparin

UFH has been widely used to treat cardiovascular disease because of its immediate onset of action. A meta-analysis showed a trend toward benefit of heparin plus aspirin compared with aspirin alone in reducing risk of myocardial infarction or death in patients with unstable angina.217 However, UFH has several unfavorable properties as an anticoagulant: unpredictable pharmacokinetics, narrow therapeutic range, and short serum half-life. These critical disadvantages of UFH led to the development of low molecular weight heparin (LMWH), a new generation of heparin. LMWH results from chemical or enzymatic depolymerization of approximately 50 residues (15 kDa) of UFH. LMWH contains oligosaccharides of less than 18 residues (<5.4 kDa), which bind to antithrombin and specifically inhibit FXa, and oligosaccharides of greater than 18 residues (>5 to 7 kD), which neutralize both FXa and thrombin (Fig. 28-5).1 LMWH exhibits less interaction with endothelial cells and plasma proteins, which leads to higher predictability of anticoagulant effect and prolonged half-life in the plasma.218 LMWH exhibits first-order kinetics with a plasma half-life approximately twofold to fourfold longer than UFH and minimal interpatient variability in effective drug levels. These properties reduce the need for continuous administration and frequent laboratory monitoring for proper dosing, resulting in a reduction of bleeding complications. LMWH also exhibits significantly fewer interactions with platelets compared with UFH. Clinical trials have shown that the incidence of heparin-induced thrombocytopenia, associated thrombotic events, and heparin-dependent IgG antibodies are significantly less common in patients treated with LMWH than in those treated with UFH.219

In addition to stimulating antithrombin activity, both UFH and LMWH provide an additional antithrombotic effect through mitigation of the TF pathway activity. Both UFH and LMWH decrease TF expression on the monocyte and endothelial cell surface, dampening the cellular procoagulant potential.220,221 In humans, both heparin preparations (intravenously or subcutaneously administered) also release TFPI from endothelial cells into the blood circulation.10,222 These agents appear to displace TFPI from endothelial cell surface glycosaminoglycans with subsequent release into the circulation and formation of heparin-TFPI complexes.223,224 Repeated or continued intravenous heparin administration depletes intravascular pools of TFPI, resulting in attenuation of the antithrombotic actions of heparin.225

FIGURE 28-5. Mechanisms of inhibitory action of unfractionated heparin (heparin) and low molecular weight heparin (LMWH) on thrombin and factor Xa. Both unfractionated heparin and LMWH bind to antithrombin (AT) through a high-affinity pentasaccharide sequence (5) that both types of heparin contain. Inhibition of thrombin (left side of figure) requires formation of a ternary complex of heparin with both antithrombin (AT) and thrombin. Unfractionated heparins have sufficient length to accomplish this but LMWHs do not. In contrast, inhibition of factor Xa (right side of figure) requires that heparin bind only to AT, which unfractionated heparin and LMWH can catalyze equally effectively through their common pentasaccharide sequences. Thus, LMWH (but not unfractionated heparin) inactivates factor Xa selectively relative to thrombin. (From Braunwald E, Zipes D, Libby P: Heart Disease, 6 ed. WB Saunders, Philadelphia, 2001, p. 2109.)
The antithrombotic efficacy of LMWH is equal or superior to UFH. Fibrinogen, prothrombin fragment 1+2 (F1+2), thrombin antithrombin complex, von Willebrand factor, TF, and TFPI manifest a similar response to UFH and LMWH among patients admitted for acute coronary syndrome. In a meta-analysis comparing LMWH with UFH in non-ST elevation acute coronary syndrome, no difference was found in the risk of myocardial infarction and death. The pooled data extracted from the ESSENCE and TIMI11B studies revealed that LMWH is superior to UFH in reducing myocardial infarction and emergency revascularization in patients with acute coronary syndrome without increasing the risk of major bleeding. LMWH was associated with a 20% reduction in death and serious cardiac ischemic events compared with UFH. In patients with ST-elevation myocardial infarction, the combination of LMWH and tenecteplase was superior to that of UFH and tenecteplase in reducing the frequency of ischemic complications.

**Warfarin**

Warfarin inhibits the synthesis of six vitamin K-dependent proteins involved in regulation of blood coagulation: prothrombin, fIX, fVII, fX, protein C, and protein S. These proteins contain 9 to 12 residues of γ-carboxyglutamic acid within the first 45 residues of their NH₂-termini. γ-Carboxylation of glutamic acids is a result of post-translational modification by a membrane-bound γ-carboxylase, and this enzyme requires the reduced form of vitamin K (KH₂) as a cofactor. γ-Carboxyglutamic acid residues in the NH₂-terminal of these proteins are required to bind to Ca²⁺ and form stable vitamin K-dependent enzyme complexes on cellular phospholipid surfaces. Warfarin inhibits reductase enzymes that are required to recycle vitamin K epoxide to vitamin KH₂ after γ-carboxylation, thereby depleting the active vitamin K cofactor (Fig. 28-6). Warfarin rarely causes skin necrosis in the first few days of administration, especially in patients with decreased levels of protein C or protein S. This is caused by mitigation of the protein C pathway resulting from rapid decrease of protein C levels compared with other vitamin K-dependent coagulation factors.

Warfarin is the most common oral anticoagulant and is used in a variety of clinical conditions, including atrial arrhythmias, ischemic heart disease, heart failure, and stroke. In a randomized clinical trial, warfarin, in combination with aspirin or given alone, was superior to aspirin alone in reducing the incidence of composite events after an acute myocardial infarction but was associated with a higher risk of bleeding.

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**FIGURE 28-6.** Vitamin K cycle and its inhibition by warfarin. Warfarin inhibits vitamin K epoxide reductase and vitamin K quinone reductase and so blocks the conversion of vitamin K epoxide to vitamin KH₂. Vitamin KH₂ is a cofactor for the carboxylation of inactive proenzymes (factors II, VII, IX, and X) to their active forms. (From Braunwald E, Zipes D, Libby P: Heart Disease, 6th ed. WB Saunders, Philadelphia, 2001, p. 2111.)
Lipid-Lowering Agents

HMG-CoA reductase inhibitors (statins) have been proven effective in both primary and secondary prevention of coronary artery disease. In addition to their lipid-lowering effects, statins appear to reduce the propensity for atherosclerosis by attenuating the extrinsic pathway of coagulation in patients with atherosclerosis. Statins reduce TF expression and activity through inhibition of Rho/Rho-kinase and activation of Akt in human macrophages and endothelial cells. Statins also inhibit cytokine-stimulated CD40 expression on these cells, further reducing TF upregulation.

High-resolution MRI has enabled long-term, noninvasive observation of atherosclerotic plaques in human patients. Prolonged statin therapy causes significant regression of established atherosclerotic lesions with markedly decreased lipid content. Observational studies reported that early initiation of statin therapy in patients with acute myocardial infarction is associated with a significant risk reduction in mortality, and discontinuation of statins after onset of symptoms completely abrogates this beneficial effect. However, a recent randomized clinical trial showed that early initiation of aggressive statin therapy for patients with acute coronary syndrome did not reduce the risk of major cardiac events compared with the placebo group, despite significant reduction in recurrent ischemic events in the first 16 weeks.

Angiotensin-Converting Enzyme Inhibitors/Angiotensin II Receptor Blockers

The efficacy of ACE inhibitors and AT1 receptor antagonists in reducing cardiovascular mortality may partially derive from inhibition of angiotensin II-mediated activation of the extrinsic pathway of coagulation. ACE inhibitors and AT1 receptor antagonists downregulate TF expression and the resultant thrombin generation in atherosclerotic plaques and monocytes. ACE inhibitors also decrease plasma TF levels and MCP-1 in patients with acute myocardial infarction, suggesting that both reduction in TF expression and inhibition of the accumulation of monocytes and macrophages may contribute to the antithrombotic effects of ACE inhibitors and AT1 receptor antagonists.

Novel Agents

Active Site Inactivated Recombinant Factor VIIa

Several types of recombinant fVIIa with inactivation of the active site have been engineered. Active site inactivated fVIIa (fVIIai) inhibits the TF/fVIIa complex-mediated FXa production by competing with endogenous fVIIa for TF, resulting in cessation of the extrinsic pathway of coagulation. However, fVIIai may not be as potent as TFPI. TFPI inhibits the FXa generation already in progress, whereas fVIIai does not.

Administration of fVIIai abolishes thrombus formation at sites of vascular injury, increases vessel patency, and inhibits cyclic flow variations caused by recurrent thrombus formation in animal models. Remarkably, antithrombotic effects of fVIIai are not complicated by bleeding. Treatment with fVIIai in rabbit balloon-injury models inhibits fibrin deposition, reduces loss of lumen, and decreases neointimal hyperplasia at the sites of vessel injury. A phase II clinical trial showed that fVIIai also significantly reduced thrombin generation and fibrin deposition in blood obtained from a human who received fVIIai.

Anti-Tissue Factor Monoclonal Antibodies

Antibodies against TF markedly reduce plaque thrombogenicity in human arterial segments. Animal studies have shown that anti-TF monoclonal antibodies prevent thrombosis and decrease occlusion of the reversion femoral artery graft. Recombinant TFPI (rTFPI) has shown favorable antithrombotic effects of fVIIai are not complicated by bleeding. Treatment with fVIIai in rabbit balloon-injury models inhibits fibrin deposition, reduces loss of lumen, and decreases neointimal hyperplasia at the sites of vessel injury. A phase II clinical trial showed that fVIIai also significantly reduced thrombin generation and fibrin deposition in blood obtained from a human who received fVIIai.

Mutant Human Tissue Factor

The substrate recognition region of TF contains two residues, Lys165 and Lys166, which are important for macromolecular substrate activation by the TF/fVIIa complex. Replacement of these two residues with alanine (K165A:K166A) in a soluble version of human TF results in a mutant hTFAA, which binds fVIIa with kinetics and affinity equivalent to wild-type sTF. However, the hTFAA/fVIIa complex shows a 34-fold reduction in catalytic efficiency for FX activation compared with the activity measured for sTF/fVIIa. A significant antithrombotic effect is displayed in arterial thrombosis when hTFAA is compared with heparin.

Phage display technology allowed affinity maturation of hTFAA to fVIIa leading to development of an hTFAA variant with an improved antithrombotic activity that is currently under preclinical investigation.

Recombinant Tissue Factor Pathway Inhibitor

Recombinant TFPI (rTFPI) is one of the most extensively studied anticoagulants. This anticoagulant significantly reduces both platelet and fibrinogen deposition on human atherosclerotic arterial segments. Animal studies, however, showed conflicting results on its effect in preventing reocclusion in models of acute arterial thrombosis. In clinical studies, rTFPI has shown favorable effects in patients with severe sepsis, but rTFPI has not been investigated in patients with acute coronary syndrome.
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**Nematode Anticoagulant Protein c2**

Recombinant nematode anticoagulant protein c2 (rNAPc2) is a potent small protein anticoagulant isolated from the hookworm *Ancylostoma caninum*. It exerts its anticoagulant effect by FX-dependent inhibition of the TF/FVIIa complex. The anticoagulant rNAPc2 forms a binary complex with FX at a site on the FXa that is distinct from the catalytic center (exo-site) and inhibits the catalytic activity of the TF/FVIIa complex to activate FX.²⁶⁵ The formation of the binary complex with circulating FX determines the pharmacokinetic profile of rNAPc2 in humans, resulting in a prolonged elimination halflife of longer than 50 hours. A phase II clinical trial has shown that subcutaneous injections of rNAPc2 were safe and effective in patients undergoing elective, unilateral total knee replacement, and the overall incidence of deep venous thrombosis was reduced by more than 50% compared with historic controls with LMWH.²⁶⁶ Another phase IIa trial demonstrated the safety of rNAPc2 and the significant suppression of thrombin generation in patients undergoing elective percutaneous coronary intervention treated with standard anticoagulant and antiplatelet therapies.²⁶⁷

**Active Site Blocked Factor IXa**

Active site blocked FIXa (FIXai) is a competitive inhibitor of FIXa assembly into the intrinsic Xase activation complex. Animal studies have shown that FIXai blocks intravascular thrombosis without substantially disturbing normal hemostasis.²⁵⁹,²⁶⁰ In a human ex vivo blood flow system, FIXai inhibits fibrin deposition.²⁷⁰ It accomplishes long-term patency and decreased aneurysmal dilation in polytetrafluoroethylene (PTFE) vascular repair, while eliminating the intraoperative morbidity of needle-hole bleeding.²⁷¹ In addition, FIXai appears to be an effective alternative anticoagulant strategy in cardiopulmonary bypass when heparin is contraindicated, affording inhibition of intravascular and extracorporeal circuit thrombosis with enhanced hemostasis in the surgical wound.²⁷² Active site blocked FIXa limited fibrin deposition within the extracorporeal circuit, comparable with the antithrombotic effect seen with heparin. In contrast to heparin, effective antithrombotic doses of FIXa significantly diminished blood loss in the thoracic cavity and in an abdominal incisional bleeding model.²⁷³ The efficacy of FIXai in atherothrombosis remains unknown.

**Factor Xa Inhibitors**

Naturally occurring FXa inhibitors include tick anticoagulant peptide (TAP) and antistatin.²⁷⁴,²⁷⁵ Both are potent and specific factor Xa inhibitors and are available in recombinant forms. Synthetic FXa inhibitors include fondaparinux, DX-9065a, and ZK-807834. A pentasaccharide, fondaparinux is an indirect FXa inhibitor that exerts its antithrombotic effects by binding to and neutralizing antithrombin. Randomized control studies have shown that fondaparinux is superior to LMWH in preventing venous thromboembolism in patients undergoing hip fracture surgery,²⁷⁶ hip-replacement surgery,²⁷⁷ and elective major knee surgery.²⁷⁸ A meta-analysis of these trials have shown a major benefit of fondaparinux over LMWH in patients undergoing major orthopedic surgery, achieving an overall risk reduction of venous thromboembolism greater than 50% without increasing the risk of clinically relevant bleeding.²⁷⁹ A randomized clinical trial has demonstrated that fondaparinux is as safe and effective as UFH in restoring coronary artery patency in patients with ST-elevation acute myocardial infarction undergoing fibrinolytic therapy.²⁸⁰ An oral, small-molecule FXa inhibitor, DX-9065a, that directly and reversibly inhibits FXa with high specificity is currently under investigation in human clinical trials.²⁸¹ In animal studies, ZK-807834 and recombinant TAP decrease reocclusion and improve patency of recanalized arteries without increasing bleeding complications compared with heparin and aspirin.²⁸²

**Thrombin Inhibitors**

Direct thrombin inhibitors interact with thrombin and block its catalytic activity on a wide range of substrates. Hirudin, originally isolated from the saliva of a medicinal leech, *Hirudo medicinalis*, is a 65 amino acid polypeptide that forms a reversible, 1:1 stoichiometric complex with thrombin. Despite important pharmacokinetic and theoretical advantages over heparin, early randomized trials failed to demonstrate a net clinical benefit of recombinant hirudin because of an excess of hemorrhagic stroke and only modest efficacy gains compared with heparin.²⁸³ Recently, however, clinical efficacy of hirudin and its derivatives has been re-evaluated by a number of clinical studies.²⁸⁴ In patients with acute coronary syndrome, hirudin is associated with less thrombin activity and slower increases in thrombin formation after discontinuation than heparin, although hirudin does not prevent generation of new thrombin.²⁸⁵ In the HERO-2 trial, bivalirudin significantly reduced the rate of adjudicated reinfarction, despite no mortality benefit, compared with UFH in patients with acute myocardial infarction treated with streptokinase. Small absolute increases were seen in mild and moderate bleeding in patients given bivalirudin.²⁸⁶ In patients with non-ST-elevation acute coronary syndrome undergoing early percutaneous coronary intervention, hirudin was shown to be more effective than heparin in reducing the incidence of death or myocardial infarction.²⁸⁷

In addition to hirudin and bivalirudin, another class of direct thrombin inhibitors is emerging as antithrombotic drugs with a wide range of indications. These synthetic, small-molecule direct thrombin inhibitors include argatroban, efegatran, inogatran, napsagatran, and melagatran/ximelagatran. The tripeptide type or peptidomimetic compounds, including argatroban, efegatran, inogatran, and napsagatran, represent a first generation of thrombin inhibitors that are pharmacokinetically characterized by relatively rapid hepatobiliary clearance and short half-lives necessitating their administration as an intravenous infusion.²⁸⁸ Ximelagatran is an oral form of direct thrombin inhibitor. After oral administration, ximelagatran is rapidly absorbed and...
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