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REVIEW

Fibrinolysis and the control of blood coagulation

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ABSTRACT

Fibrin plays an essential role in hemostasis as both the primary product of the coagulation cascade and the ultimate substrate for fibrinolysis. Fibrinolysis efficiency is greatly influenced by clot structure, fibrinogen isoforms and polymorphisms, the rate of thrombin generation, the reactivity of thrombus-associated cells such as platelets, and the overall biochemical environment. Regulation of the fibrinolytic system, like that of the coagulation cascade, is accomplished by a wide array of cofactors, receptors, and inhibitors. Fibrinolytic activity can be generated either on the surface of a fibrin-containing thrombus, or on cells that express profibrinolytic receptors. In a widening spectrum of clinical disorders, acquired and congenital defects in fibrinolysis contribute to disease morbidity, and new assays of global fibrinolysis now have potential predictive value in multiple clinical settings. Here, we summarize the basic elements of the fibrinolytic system, points of interaction with the coagulation pathway, and some recent clinical advances.

1. Introduction

Platelets are activated upon contact with subendothelial matrix proteins, including collagen, von Willebrand factor, and fibronectin, in response to vascular injury [1]. Platelet activation leads to exposure of cell surface anionic phospholipids, which serve as a nidus for the assembly of procoagulant proteins. In the ensuing activation of the coagulation cascade, a sequential series of serine protease-mediated cleavage events, thrombin is activated from its zymogen prothrombin [2]. Active thrombin can then catalyze the polymerization of fibrin by cleaving small peptides from two of its three subunits. Polymerization converts soluble fibrinogen into insoluble fibrin, which stems the flow of blood, thus achieving "hemostasis," the prevention of major blood loss [3]. As the clot or "thrombus" forms, circulating red blood cells, white blood cells, and platelets become incorporated into its structure. In addition, fibrin becomes cross-linked through the action of factor XIIIa, which is also activated by thrombin, and provides further structural stability [4]. Upon healing of the injured blood vessel, the effete thrombus is lysed through the action of plasmin. Plasmin is generated from the zymogen plasminogen on the surface of the fibrin clot, or on cell surfaces, by either tissue plasminogen activator (tPA) or urokinase (uPA) [5]. Proteolysis of fibrin gives rise to soluble fibrin degradation products (FDPs), some of which have immunomodulatory and chemotactic functions. The coagulation and fibrinolytic systems are highly regulated and inter-related through mechanisms that insure balanced hemostasis.

2. Fibrin formation and clot structure

Fibrinogen, a soluble 340-kDa protein, circulates in whole blood at concentrations of 2–4 mg/mL [6]. It consists of two sets of three distinct disulfide-linked polypeptide chains (Aα, Bβ, and γ), whose synthetic programs are directed by three separate genes on chromosome 4. Thrombin's major molecular target is fibrinogen, which is converted to fibrin monomers as thrombin removes N-terminal fibrinopeptides A and B. The resulting monomer is a disulfide-linked trinodular protein whose N- and C-termini converge at the E- and D-nodules, respectively.

Assembly of fibrin fibers then proceeds in a stepwise fashion. After an initial lag phase, release of fibrinopeptide A encourages protofibril formation by the lateral aggregation of fibrin fibers, wherein the E domain of one homodimer interacts with the D domain of a second to generate a half-staggered, overlapping fibrillar pattern within the developing thrombus [6]. Fibrin is cross-linked at lysine residues by factor XIIIa and forms fibrillar aggregates, which, together with platelets and red blood cells, provide structural integrity to the growing thrombus [7]. Turbidity and circulatory flow assist in fibrin polymerization and protofibril assembly by orienting the fibers as the growing thrombus forms [8–12].

Many factors, including local calcium concentration, pH, and platelet numbers, affect clot stability [6]. Stability is also based partly on fibrin fiber diameter, and the geometry of the fibrin network. Local thrombin concentration also impacts clot structure, as higher thrombin concentrations generate more stable clots [6,11,13]. Fragile clots are more

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susceptible to fibrinolysis and bleeding, whereas firm clots are more resistant, but may promote thrombosis [14–16]. For example, hemophilia patients have both spontaneous bleeding and poor clot formation resulting from impaired peak thrombin generation; the resulting thrombi are porous and more susceptible to fibrinolysis [17–19]. The variables that affect fiber architecture are ultimately important for fibrinolysis, since both fiber size and arrangement impact tissue plasminogen activator (tPA) binding and rates of fibrinolysis [20–23].

γ′-Fibrinogens are γ-chain splice variants that compromise approximately 8–15% of γ-fibrinogen (γA/γ′), compared to the more common fibrinogen γA/γA. γ′-Fibrinogens result in the formation of thinner fibers with increased branching [24]. Epidemiologic data initially indicated that γ′-fibrinogen isoforms were elevated in patients with arterial thrombosis, but more recently both human and murine models suggest a relative antithrombotic role for γA/γ′, possibly as a result of thrombin sequestration [25,26]. Thrombus formation depends upon not only the total fibrinogen concentration, but also the isoform composition of the fibrinogen pool.

Clot structure, therefore, reflects the complex interplay of many factors ranging from polymorphisms in fibrinogen itself, to the efficiency of thrombin generation, the reactivity of associated cells, such as platelets, and the biochemical milieu. These components define fibrin clot architecture, which is a key determinant of the efficiency of clot lysis [27].

3. Regulation of fibrinolysis

Like the coagulation cascade, fibrinolysis is tightly controlled by a series of cofactors, inhibitors, and receptors [5]. Plasmin is the primary fibrinolysin, and is activated from plasminogen by either of two primary serine proteases, tPA and uPA. Whereas tPA is synthesized and released by endothelial cells, uPA is produced by monocytes, macrophages, and urinary epithelium. Both activators have exceedingly short half-lives in circulation (4–8 minutes) due to the presence of high concentrations of specific inhibitors, such as plasminogen activator inhibitor-1 (PAI-1). Compared to tPA, uPA has lower affinity for plasminogen, does not require fibrin as a cofactor, and, under normal conditions, appears to act mainly in extravascular locations. Both tPA and uPA are cleared by the liver after forming complexes with a low density lipoprotein (LDL)-receptor-like protein [28]. Because plasmin increases activator activity by converting single-chain tPA and uPA to their two-chain counterparts, plasminogen exerts positive feedback on its own activation [29–31].

Inhibitors are also important to prevent excess unregulated plasmin or plasminogen activator activity. Circulating plasmin and plasminogen activators are neutralized by serine protease inhibitors, or serpins, which are present in excess concentrations [32]. Serpins form covalent complexes with their unique target enzymes that are subsequently cleared from the circulation. The three serpins most important in fibrinolysis are plasminogen activator inhibitor-1 (PAI-1), plasminogen activator inhibitor-2 (PAI-2), and α2-antiplasmin (A2AP). Plasmin and A2AP bind with 1:1 stoichiometry, whereupon both become inactive. When plasmin is bound to fibrin, however, it is protected from A2AP inhibition, allowing for fibrinolysis to proceed [33]. Similarly, the plasminogen activators tPA and uPA are rapidly inhibited by PAI-1, which is released into the circulation from endothelial cells, platelets, and other cells [34]. PAI-1 is upregulated by a large number of proinflammatory cytokines as well [5]. In pregnancy, PAI-2 is also a major tPA and uPA inhibitor, and its concentrations increase as the pregnancy progresses. Deficiencies in PAI-2 have been associated with adverse pregnancy outcomes [35,36]. Other non-serpin plasmin inhibitors include α2-macroglobulin, C1-esterase inhibitor, and members of the contact pathway of the coagulation cascade, which also play minor roles in plasmin inhibition.

Thrombin activated fibrinolysis inhibitor (TAFI) is a non-serpin fibrinolysis inhibitor that is activated by thrombomodulin-associated thrombin. TAFI is a carboxypeptidase that removes C-terminal lysine and arginine residues on fibrin, thereby decreasing the number of available plasminogen binding sites, slowing plasmin generation, and stabilizing clots. TAFI is found at reduced levels in hemophilia patients as a result of impaired thrombin burst, and leads to increased fibrinolysis [37,38].

3.1. Thrombus-based fibrinolysis

Fibrinolysis is a highly regulated enzymatic process that prevents unnecessary accumulation of intravascular fibrin and enables the removal of thrombi. Fibrin surfaces are key activation sites for fibrinolysis that modulate the binding of plasminogen and plasmin [29]. Fibrin-bound tPA, for example, shows an approximately 500-fold increase in catalytic efficiency of plasminogen activation compared to tPA in the fluid phase [30]. Similarly, plasmin is protected from inhibition by A2AP upon binding to fibrin, while initially fibrin-bound A2AP protects the clot from fibrinolysis [39–41]. Because both fibrin and fibrinogen increase conversion of plasminogen to plasmin, they facilitate their own destruction [42,43]. Fibrin clearance is also accelerated by providing new binding sites for plasminogen, as C-terminal lysine residues become exposed at an increasing rate during fibrinolysis.

3.2. Cell surface fibrinolysis

While plasmin, tPA, and uPA are all neutralized by soluble circulating inhibitors, the surfaces of endothelial cells and the fibrin thrombus offer a safe haven for preserving their fibrinolytic activity. Several cell surface molecules, including a variety of plasminogen receptors, the uPA receptor (uPAR), and the annexin A2 complex bind plasminogen and/or its activators on endothelial cells, monocytes, and many other cell types [29]. Some receptors, such as uPAR and the transmembrane plasminogen receptor (PlgR-KT) may modulate additional non-fibrinolytic functions, some as diverse as cell-matrix adhesion and catecholamine release [44–46].

Annexin A2, an important component of cell-based fibrinolysis, is a member of the annexin family of calcium-binding proteins that fulfill diverse physiologic functions [47–50]. On the surface of endothelial cells and monocytes, annexin A2 forms a heterotetrameric complex with another protein, S100A10 (also known as p11); the complex serves as a profibrinolytic receptor that binds plasminogen and tPA, but not uPA [51]. The (annexin $A2_2$-$S100A10_2$) complex strongly promotes the tPA-dependent activation of plasmin independently of fibrin [47,52,53].

Inhibition of the annexin A2 complex's function may increase thrombosis risk by impairing fibrinolysis. High-titer antibodies directed against annexin A2 have been observed with increased frequency in patients with antiphospholipid syndrome and a history of thrombosis, and also in a cohort of patients with cerebral venous thrombosis [54,55]. Polymorphisms in annexin A2 have also been associated with vascular occlusion in patients with sickle cell disease [56,57]. Conversely, abnormally high levels of annexin A2 are expressed by blast cells in acute promyelocytic leukemia (APL) and appear to contribute to increased fibrinolysis and bleeding (Fig. 1) [58,59]. On APL cells, annexin A2 probably increases fibrinolysis in concert with protein p11, which is also upregulated in an autonomous APL cell line [60].

4. Fibrin degradation products

Fibrin degradation products (FDPs) begin to form as plasminogen is activated and plasmin begins to degrade the thrombus. Multiple FDPs, including fibrinopeptide B and other fibrin degradation monomers and dimers are released [61–63]. When fibrin polymers are cleaved by plasmin at the D fragment site, the resulting D-dimer fragment reflects