Blood coagulation in immunothrombosis—At the frontline of intravascular immunity

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A B S T R A C T

While hemostasis is the physiological process that prevents blood loss after vessel injury, thrombosis is often portrayed as a pathologic event involving blood coagulation and platelet aggregation eventually leading to vascular occlusion and tissue damage. However, recent work suggests that thrombosis can also be a physiological process, termed immunothrombosis, initiated by the innate immune system providing a first line of defense to locally control infection. Fibrin forms the structural basis of immunothrombotic clots and its assembly involves the concerted action of coagulation factors, platelets and leukocytes.

Here, we summarize the cellular and molecular events that initiate fibrin formation during the innate immune response and discuss how aberrant activation of these pathways fosters pathologies associated with thrombosis, including disseminated intravascular coagulation and atherothrombosis.

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1. Introduction: immunothrombosis — an ancient and conserved host-defense mechanism

Vertebrates possess a closed circulatory system pumping blood through a network of vessels carpeted by an endothelial cell barrier that covers a surface area of about 7 square meters in adult humans [1]. Vascular leakage is a threat to life and maintenance of vascular integrity is thus crucial for host survival. Hemostasis is the physiological response to vascular injury that prevents excessive bleeding and maintains vascular integrity. In mammals two major mechanism are involved in hemostasis — the initiation of blood coagulation and the activation of platelets. Consequently, genetic or pharmacological perturbations of both systems cause clinically relevant bleeding disorders and the role of clot formation in mammals as a highly specialized process to maintain intravascular blood volume is well established [2,3].

In a Darwinian world, organisms live under a constant threat of pathogenic invaders, and vascular damage not only causes blood loss, but also presents a port of entry that has to be guarded instantaneously. Linked to the urgency of the hemostatic response platelets are among the first cells that encounter these sites of injury and in concert with rapidly forming fibrin establish the first line of host defense. Indeed, an ancient link between hemostasis and host defense was suggested by comparative studies analyzing more primitive blood of invertebrates. E.g. the Atlantic horseshoe

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2. The coagulation cascade

In mammals blood is pumped through a closed vascular circuit to perform its multiple physiological functions throughout the body, including oxygen supply and immune surveillance. While fluidity of blood is central for its energy-efficient transportation, it becomes detrimental when a vessel is injured and blood starts leaking. Platelets and fibrin, which both mediate the hemostatic response, instantaneously form a clot to seal the leak and to maintain vascular integrity. Fibrin is the major, glue-like end-product of the coagulation system and forms a provisional, extracellular matrix consisting of a branched, three-dimensional network of strained fibers.

Fibrin formation is initiated by the sequential activation of blood-borne serine proteases and their cofactors (recently reviewed in [16]). Briefly, conversion of fibrinogen into fibrin requires the proteolytic action of thrombin (factor IIa), a central mediator of hemostasis. Thrombin circulates as an inactive zymogen (prothrombin or factor II) and its activation requires the assembly of the prothrombinase complex comprising the serine protease activated factor X (FXa) and the cofactors activated factor V (FVa), calcium as well as negatively charged phospholipid membranes. Two coagulation pathways have been described to activate the prothrombinase complex. The intrinsic or contact pathway requires the activation by contact with a negatively charged surface and involves kininogen, kallikrein and coagulation factors XII, XI, IX, VIII and V, while the extrinsic pathway involves tissue factor exposed to the circulation and coagulation factor VII. Even though each pathway can be measured separately in clinical coagulation tests by the activated partial thromboplastin time (intrinsic pathway) and the prothrombin time (extrinsic pathway), respectively, it is now well accepted that this artificial separation does not fully reflect the in vivo situation [16]. Based on our better understanding of the molecular events triggering fibrin formation in vivo, an updated view of the coagulation cascade emphasizes the crucial importance of tissue factor as the major initiator of coagulation (see Fig. 1). Tissue factor is a membrane bound glycoprotein expressed by vessel wall cells that are not exposed to blood (such as adventitial fibroblasts) and by non-vascular tissue cells [17]. The intact endothelial barrier spatially segregates tissue factor and circulating blood clotting factors ensuring that the coagulation cascade is only initiated when the vessel wall is disrupted. Exposed to blood, tissue factor interacts with a blood–based coagulation factor termed factor VIIa and forms the extrinsic tenase complex (TF:FVa) on phospholipid surfaces of the tissue factor bearing membrane. TF:FVila than activates factor IX (FIXa) as well as factor X (FXa). As part of the prothrombinase complex the latter initiates the conversion of small amounts of thrombin, sufficient enough to trigger the amplification phase of coagulation by activating platelets, as well as coagulation factors V (to FVa), VIII (to FVila) and XI (to FxIa). This produces additional FIxa which assembles with FVIIa on platelet membranes to form the intrinsic tenase complex (FIXa:FVIIa), thereby increasing the efficiency of FX activation. Consequently, the amplification loop of coagulation increases both the amount of FXa as well as FVa assembled on the surface of activated platelets (prothrombinase complex) and thereby triggers a burst of thrombin. In order to prevent uncontrolled or disseminated coagulation, the organism developed natural “breaks” to self-limit the above-mentioned processes to the site of injury. As such, at least three natural anticoagulants have been described including the tissue factor pathway inhibitor (TFPI), vitamin K-dependent protein C and antithrombin [18]. Indeed when these breaks are released, the risk of disseminated intravascular coagulation triggered by tissue injury as well as invading pathogens increases significantly [19,20,21].

3. Structural organization of fibrin networks

Fibrin clot formation is a multistep process resulting in the organized polymerization of the soluble precursor fibrinogen, a 340 kDa glycoprotein consisting of 2Aα-, 2Bβ-, and 2γ-chains, that are linked by 29 disulfide bonds to build a dimeric structure [22]. The initial conversion of fibrinogen into fibrin monomers requires limited proteolysis by the serine-protease thrombin, which cleaves two fibrinopeptides (Fp), FpA and FpB, from the N-termi of the Aα- and Bβ-chains, respectively. This cleavage results in the exposure of cryptic motifs facilitating the intermolecular half staggered end-to-end assembly of fibrin monomers into double stranded prototibrils, as well as the subsequent lateral aggregation of these.