Blood Coagulation as an Intrinsic Pathway for Proinflammation: A Mini Review

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Abstract: Blood coagulation could be recognized as intrinsic inflammation. The coagulant mediators (FVIIa, FXa, thrombin (FIIa), FXIIa) and fibrin(ogen) activate cellular signaling, eliciting the production of cytokines, chemokines, growth factors, and other proinflammatory mediators. Hypercoagulability with elevated coagulant mediators would certainly trigger hyper-inflammatory state not to mention about the direct hypercoagulable actions on thrombosis, and platelet and complement activations, all of which contribute to inflammatory events. Furthermore, anticoagulant’s anti-inflammatory effects readily reinforce the proposal that blood coagulation results in inflammation. The observations on protease activated receptor (PAR) activation and PAR antagonists modulating inflammation are also in line with the concept of coagulation-dependent inflammation.

Keywords: Blood coagulation, hypercoagulability, tissue factor, coagulant mediator, anticoagulant, inflammation, cytokines, protease-activated receptors, complement, thrombosis, cardioprotection.

1. INTRODUCTION

Inflammation plays a “diverging-converging” role in widely involving a broad spectrum of disease statuses and complications regardless of their pathogeneses. For instance, depression has even been recognized an inflammatory state [1]. Heterogeneous chronic obstructive pulmonary disease readily manifests as respiratory inflammation [2] somewhat similar to asthma outcome.

Inflammation could be generally associated with elevated proinflammatory network involving cytokines (e.g., TNFα, IL-1β, IL-6, etc.), chemokines (CXC’s, CCL’s, etc.), adhesion molecules (MCP-1, ICAM, VCAM, selectins, etc.), growth factors (e.g., VEGF, PDGF, GM-CSF, etc.), and other inflammatory mediators (e.g., CRP, PGE2, LTs, PAF, histamine, matrix metalloproteinases (MMP), bradykinin (BK), calpain, complements, ET-1, ATII, etc.). The pleiotropic effects of these inflammatory mediators include activating T/B cell maturation/differentiation, non-immune cells, cellular signaling, and gene expressions. Inflammation damages surrounding tissues, contributes to angiogenesis/tumorigenesis, and manifests as many symptoms. Accordingly, combating inflammation becomes strategic approaches for relieving many disease complications. Anticytokine approaches, including reception suppression by soluble cytokine receptors and/or receptor analogous, offer broad clinical benefits to human inflammatory diseases [3] such as asthma [4], autoimmune disorders [5] (e.g., rheumatoid arthritis (RA), Crohn’s disease, and psoriasis), and many others. Anti-VEGF Ab shows antagonism against RA by blocking VEGF reception and cytokine (TNF-α and IL-6) production [6]. Anti-P-selectin antibody attenuates inflammation [7].

The source of inflammation is heterogeneous. Clinical studies readily demonstrate the close relationship of infection with inflammation. In most cases, inflammation in response to infection is essentially part of innate and adaptive immune systems. There is a perception that inflammation is most likely mediated by Toll-like receptors (TLR) [for reviews, see ref. 8, 9] receiving signals from either exogenous pathogen or endogenous danger-associated molecular patterns as a consequence of diverse infection or tissue injury/damage, respectively. Through MyD88-dependent or independent cellular activation of transcription factors (e.g, AP-1, NFκB, and IRF3), proinflammatory genes are upregulated thereby resulting in local and/or systemic inflammation. Accordingly, TLR7/9 antagonists for immune-mediated inflammatory diseases have been reported [10].

Non-infectious conditions such as oxidative stress, arachidonate/eicosanoid metabolism, and many others could also lead to inflammatory responses. This mini-review addresses that blood coagulation triggers inflammation. Hypercoagulability accompanied by elevated clotting factors (e.g., FVIIa, FXa, FIIa, and FXIIa) and fibrin clot over-production [11-13] is responsible for cellular activation, which results in the elevated productions of cytokines, adhesion molecules, and many other inflammatory mediators. The evidence revealing the antiinflammatory property of anticoagulation further supports such coagulation-dependent inflammation. In addition, direct hypercoagulable consequences (e.g., thrombosis and platelet and complement activations) readily contribute to inflammation. Thus, blood coagulation could be considered as endogenous/intrinsic local or systemic inflammation.

2. OVERVIEW OF BLOOD COAGULATION PATHWAYS

Blood coagulation is a primary biological phenomenon, as a self defense system stopping bleeding during injury, in
mammals and other animals. However, often excessive or uncontrolled clot production leads to thrombosis shutting off blood flow that supplies oxygen and nutrients to cells. The resulting cell death manifests as stroke or heart attack. Coagulation has also been recognized an independent risk factor for atherosclerosis; fibrin stimulates plaque growth. Hypercoagulability shares similar risk factors with thrombosis involving congenital/acquired genetic deficiencies, diverse pathological conditions, lifestyle transitions, medical procedures, and aging [for review, see ref. 12]. Hypercoagulable state could also result from the defect in fibrinolytic system involving plasminogen activator (PA)-plasmin activation [for review, see ref. 14], which is beyond the focus of this review.

There are two coagulation pathways essentially proceeding as extracellular signaling cascades. The inducible extrinsic pathway plays an integral role [15], while the intrinsic pathway is constitutive facilitating blood coagulation merging with the extrinsic pathway at the point of tenase. The extrinsic but not intrinsic pathway is responsible for the initiation of thrombin generation and fibrin production [16].

2.1. Extrinsic Pathway

Integral membrane glycoprotein: tissue factor (TF; CD142) initiates the extrinsic pathways, which is susceptible to in vivo upregulation by inflammation [for review, see ref. 17], vascular injury (i.e., protein disulfide isomerase) [16, 18, 19], or advanced glycated end products under hyperglycemia. TF expression is mediated by the activations of signaling kinases (e.g., PTK, PKC, MAPK) and transcription factors (e.g., AP-1, Egr-1, NFkB) while negatively correlating to intracellular cAMP level [for review, see ref. 17]. Nitric oxide (NO) synthases and cyclooxygenase (COX)1/2, however, seem to have nothing to do with TF expression [17]. In some clinical or pathological conditions, TF shed from vascular cells known as circulating plasma TF associated with microparticles also results in hypercoagulability and increased thrombotic risk [20, 21]. The microparticles involving selectin glycoprotein ligand 1 (PSGL-1) are captured by P-selectin to the sites of activated endothelium.

A series of zymogen activations take place on phospholipid-rich cell surfaces in a vitamin K and Ca+2-dependent fashion [12, 16]. Upon FVII binding, TF-dependent FVII activation leads to FVIIa formation; this active serine protease triggers sequential proteolyses of FX and prothrombin to generate active serine proteases: FXa and thrombin (FIIa), respectively. FIIa is responsible for cleaving fibrinogen (FBG); the resulting fibrin is further polymerized and consequently cross-linked by FXIIIa, thereby producing insoluble blood clots (Fig. 1A). There are accessory loops for promoting the propagation of clotting. The initial FIIa formation activates FV and FVIII to form FVa and FVIIIa respectively, which activates tenase for fueling blood coagulation in the absence of TF [for review, see ref. 16].

2.2. Intrinsic Pathway

In the complementary intrinsic pathway (contact system) [for review, see refs. 22-24], the coagulation cascade involves high molecular weight kininogen (HK)-dependent prekallikrein (PKK)/kallikrein (KK) regenerating cycle for FVIIa-dependent FXII activation (Fig. 1B). The resulting FVIIa subsequently activates FIX following with the activation of FIX so that the intrinsic tenase shunts into the major extrinsic pathway in contribution to FXa generation [16]. The role of FXII (Hageman factor) in thrombogenesis, however, remains largely unclear.

Its upregulation is associated with many infectious and inflammatory conditions including severe *falciparum malaria* [25], *meningococcal* septic shock [26], sepsis [27], *Rhinovirus* infection [28], *Staphylococcus aureus* infection [29], LPS [30, 31], and complement activation [32]. Others such as pregnancy [33], estrogen [34], and artifact thawing/freezing [35] also activate contact coagulation.

3. BLOOD COAGULATION- INTRINSIC INFLAMMATION

The contribution of blood coagulation to inflammation could result from coagulant mediators, thrombosis, complement activation, and platelet activation. The close clinical links of coagulation with inflammation have been reported in sepsis [36], DIC [37], inflammatory bowel diseases [38], lung diseases [39] including acute lung injury, acute respiratory distress syndrome and pneumonia [40]. Clinical studies implying the relationship of coagulation with inflammation showed that natural anticoagulant (e.g., activated protein C (APC), antithrombin (ATIII)) deficiencies are often associated with sepsis [41], DIC consequences [42], and inflammation [43]. C-1 inhibitor deficiency is also susceptible to inflammation and septic shock [44], and the deficiency is often associated with increased vascular permeability [45], a biomarker for inflammation.

Furthermore, anti-TF Ab shows anti-septic action [46], which lays the foundation for coagulation-dependent inflammation. TF and FVIIa receptor/ligand interactions induce proinflammatory effects in macrophages [47]. Convincingly, the role of TF in proinflammation comes from the demonstration that recombinant soluble TF (sTF1-219) readily induces inflammation in vivo arthritis model with elevated plasma IL-6 and paw swelling, which is accompanied by fibrin production and platelet activation [48]. In addition, FVII deficiency protects against acute inflammation [49], while elevated plasma level of FVIIa shows significant correlations to CRP and IL-6 [50]. Administration with recombinant soluble TFVIIa enhances IL-6 and -8 productions in healthy human subjects [51].

3.1. Proinflammatory Mediator Production by Coagulant Mediators

Fig. (1) depicts that both the extrinsic (A) and intrinsic (B) coagulation elicit inflammatory events. Unlike TLRs engaging in the majority of infection-triggered inflammation [for review, see ref. 8, 9], protease activated receptors (PAR) are in the interface between the extrinsic coagulant signals and intracellular activations. Active serine proteases such as coagulant mediators (FVIIa, FXa, and FIIa) readily cleave and activate PARs, G-protein coupled receptors. As the result of PAR activation, intracellular signaling components and transcription factors are upregulated, all of which are responsible for enhanced cytokine production. PARs
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Transmit the coagulant signals for the production of proinflammatory mediators, while TLR-4 transduces fibrin(ogen) signals (Fig. 1A). PAR-1 conveys FIIa and FXa signals, PAR-2 delivers FVIIa and FXa signals, PAR-3 mediates FIIa and FXa signals, and PAR-4 transduces FIIa signal.

Fig. (1). Coagulation-dependent inflammation. (A) TF-initiated extrinsic coagulation (left panel) proceeds as extracellular signaling and results in the generation of active serine protease (coagulant mediators: FVIIa, FXa, FIIa) derived from their corresponding zymogens. FBG is cleaved by FIIa to produce fibrin that is polymerized and crosslinked to yield insoluble blood clots. Through cell receptors (PARs and TLR) on plasma membrane, the signals from the coagulant mediators as well as fibrin mediate diverse intracellular activation and the production of proinflammatory mediators (right panel) including cytokines, adhesion molecules, growth factors, etc. (B) The contact system (intrinsic coagulation) consists of a HK/PKK/KK regenerating cycle for FVIIa-dependent FXII activation. gC1qR/p33 protein receptor is involved in KK production and subsequent BK release from PKK and HK, respectively. KK triggers complement component activation, while BK activates cytokine production and intracellular signaling for proinflammation.
A receptor protein, gC1qR/p33 [52], initiates PKK activation to KK for complement activation, and B-1/B-2 receptors [53] are responsible for the transduction of bradykinin (BK) proinflammation (Fig. 1B).

### 3.1.1. PAR Activation Leading to Inflammation

PAR activating peptides elicit a broad spectrum of inflammation, which is consistent with the notion of coagulation-dependent inflammation.

#### 3.1.1.1. PAR-1 Activation

An activating peptide (TRAP) enhances IL-6 [54], PGE2 [55], PGDF or P-selectin expression [56]. Another activating peptide (SFLLRN) induces the production of IL-6 [57], -8 [58], iNOS [59], PGE2 [57], MCP-1 [58], NO [60], or P-selectin [61]. SFLLKKNPNDKYEF elicits the expression of ICAM [62] and VEGF [63]. A recent study has reported that activated protein C (APC) activates PAR-1 to induce MCP-1 expression in EC [64].

#### 3.1.1.2. PAR-2 Activation

Trypsin or trypsin elicits the production of TNF-α [65], IL-1β [65], IL-6 [65-67] or IL-8 [67, 68] and increases [Ca^2+], [68]. Its signaling also activates Erk [67], AP-1 [67], c-Jun [67], p38 MAPK [67], or NFκB [68]. Apart from enhanced PI hydrolysis [69], a PAR-2 activating peptide (SLIGKV) not only elicits [57] IL-6, -8 and PGE2, but also activates p38 MAPK [70] and MEK/ERK [71] to upregulate TNF-α secretion [72], PGE2 production [55], and Erk activation [73]. Enhanced NO production [60] or P-selectin expression [74] by SLIGRL (activating peptide) accounts for muscle relaxation or leukocyte rolling, respectively. Agonist proteinase-3 (PR3) enhances IL-8, MCP-1 and ICAM-1 expression [75]. An in vivo comparison of null mutant (PAR-2 -/-) with wide-type (PAR-2 +/+) mice consistently indicates that PAR-2 is responsible for the induction of IL-6, ICAM-1 and E-selectin expression [76]. Diverse PAR-2 actions also go beyond this review’s inflammation focus, involving pigmentation, vasodilation, pain/itch, IBS transmission, etc.

#### 3.1.1.3. PAR-3 Activation

A peptide (TRFGRAP) [77] induces Erk1/2 activation and [Ca^2+]i, while SFNNGP [55] slightly elicits PGE2 release.

#### 3.1.1.4. PAR-4 Activation

A peptide (GYPGQV) [57, 78] elicits TNF-α, IL-6, -8 or PGE2 production. GYPGKF enhances PGE2 production [55], [Ca^2+], and platelet aggregation [79]. AYPGKF [80] activates p38 MAPK, PLC, and Src.

#### 3.1.2. FIIa

In TF-expressing cells, FIIa elicits VEGF [81] and activates PAR-2 to enhance SMC migration [82], MAPK phosphorylation, and [Ca^2+2] mobilization [83]. Enhanced IL-8 expression in response to FIIa is mediated by PAR-2, facilitating cell migration [84]. In addition, PAR-2-mediated FIIa signaling stimulates ET-1 synthesis [85].

#### 3.1.3. FXa

FXa signaling elicits IL-6 [86, 87], IL-8 [86] or MCP-1 [55, 86] expression, which is PAR-1/3-dependent. PAR-2-dependent FXa signaling induces the expression of IL-6 [87]-8 [86], PDGF or MCP-1 [86] as well as Erk1/2 activation [73].

#### 3.1.4. FIIia

FIIia upregulates the production of IL-6 [52, 86, 88-90], IL-8 [86], TGF-β [91], MCP-1 [86, 87, 89], PDGF [90], bFGF [90], ICAM-1 [62], P-selectin [91], or VEGF [63, 92]. PAR-1-dependent FIIa signaling enhances proinflammatory actions including NFκB activation [62], intracellular Ca^2+ mobilization, Erk1/2 phosphorylation [92], iNOS [57], COX-2 [94], MMP-9 [95], and PI hydrolysis [69]. FIIa-induced PAR-1 signaling of IL-6, IL-8, MCP-1, PGE2 production and NFκB activation is apparently sphingosine kinase 1-dependent [96].

#### 3.1.5. Fibrin

TLR 4 is proposed to mediate FBG pro-inflammation [97] including IL-6 and MCP-1 production [88]. Fibrin and its fragments elicit IL-1β [98], IL-6 [99], IL-8 [100] and ICAM [100] expression through undefined mechanism(s).

#### 3.1.6. FXIIa

Proinflammatory effects of FXII activation are largely mediated by KK and BK generation [101]. In the PKK/KK/HK regenerating loop, FXII undergoes autoactivation to FXIIa that further activates PKK to KK [102] (Fig. 1B), subsequently releasing BK. gC1qR/p33, a membrane-bound receptor protein binding to HK, initiates PKK activation to KK in the presence of Zn^2+ and FXII [52, 103], thereby serving as a molecular bridge between contact activation and complement systems.

#### 3.1.6.1. KK-Dependent Complement Activation

Proinflammatory KK directly activates not only complement components C3 and C5 [104], but also C1 esterase (Fig. 1B) leading to inflammation via the classical complement activation [104, 105]. As the result of complement activation, it leads to NFκB activation, the transcription and expression of adhesion molecules (VCAM-1, ICAM-1 and E-and P-selectins), production of IL-8 and MCP-1, platelet activation, and platelet-leukocyte aggregation, all of which represent inflammation [for review, see ref. 106]. KK also cleaves HK to liberate BK [105], another proinflammatory mediator (see below section 3.1.6.2.). It, however, remains largely unknown whether PARs mediate KK proinflammatory action.

#### 3.1.6.2. BK Release

Proinflammatory mediator BK (Arg-Pro-Pro-Gly-Phes-Ser-Pro-Phe-Arg) is known as a major contributor to the innate inflammatory response. Derived from HK through the direct catalysis by KK (Fig. 1B), BK is responsible for vasodilatation, increased vascular permeability, and inflammatory pain [104, 107-109].

The involvement of BK in inflammatory processes has been demonstrated in disease status [for review, see ref. 109]. BK proinflammation is largely mediated by its inducible B1 receptor or constitutive B2 receptor [53]. More specifically, B2-receptor is responsible for swelling and pain, while B1 receptor is specific in hyperalgesia. These G protein coupled receptors initiate complex intracellular signaling cascade involving adenylate cyclase, PLC, Ca^2+...
signal, MAPKs, etc., leading to NO and prostaglandin production. BK through B1 receptor promotes ICAM/VCAM expression [110]; B1 receptor activation also induces TNF-α and IL-1β release [111]. Through B2 receptor, BK induces COX-2 expression in aortic vascular smooth muscle cells [112] involving the activation of MAPK p42/p44, PKC, and NOS. COX-2 products (e.g., TXA2, PGE2) have inflammatory properties. BK via B2 receptor induces MMP-2 production [113].

Concerning intracellular activation, BK generally upregulates MAPK [114-116], NOS [117], and JAK/STAT signaling [118]. BK induces IL-6 [119] and IL-8 [120] expression, which is mediated by ERK1/2 and p38 MAPK activation in AP-1-dependent fashion [119].

3.2. Complement Activation and Inflammation

KK in the intrinsic pathway activates complement components (Fig. 1B; also see section 3.1.6.1). In addition, FIIa could directly activate C5 and C3 [121]. Platelet activation resulting from coagulation also engages in complement activation (see section 3.3.).

Complement activation readily engages in virtually all phases of an acute inflammatory reaction including the increase in vascular permeability, extravasation of leukocytes, and chemotaxis. Complement activation presents inflammation [106], eliciting an array of proinflammatory mediators including upregulated mRNA levels of CRP, TF, COX-2, TNF, E-selectin, ICAM, VCAM, 1αβ3, IL-1α, IL-1β, IL-8, and PAI-1. C1q is able to induce the production of IL-8, while C5a elicits IL-3 and MCP-1 production. C5b-9 induces IL-6/IL-8 production and the expression of AP-1 and NFκB.

3.3. Platelet Activation and Inflammation

As a direct consequence of blood coagulation, platelet activation is primarily mediated by FIIa [122]. Platelet activation [122, 123] per se releases a broad spectrum of cytokines, adhesion molecules, growth factors, chemokines, and other inflammatory mediators. For cell neighboring effects, activated platelets result in activations of vascular cells such as endothelial cells, smooth muscle cells, lymphocytes, etc., which leads to local/systemic inflammation.

Apart from FIIa reception, other platelet receptors such as GPIb/IX/V, P-selectin, PSGL-1, CD40, gC1qR, and αIIbβ3 integrin participate in the progression of inflammatory conditions [124]. Interestingly, platelet activates C5 [125], while C3 activation is mediated by C1q-dependent classical pathway [125]; all represent inflammatory events [106, 121]. In addition, platelet activation results in aggregation and fibrin recruitment for thrombosis [16] that also contributes to inflammation (see below section 3.4.).

3.4. Thrombosis and Inflammation

The close association of thrombosis with inflammation has previously been reviewed by Esmon [126], which is in line with hypercoagulability presenting the high tendency of thrombogenesis [13] and thrombotic contributions to inflammation [for review, see ref. 127]. In these regards, platelet activation and aggregation play roles in inflammation as described in the above section 3.2. & 3.3. The notion of profibrinolysis via plasminogen activation showing antiinflammation could be in further agreement with thrombosis-dependent inflammation [128].

4. ANTAGONISM AGAINST INFLAMMATION DERIVED FROM BLOOD COAGULATION

Blood coagulation could become a target for antiinflammation (Table 1). Convincing evidence demonstrates natural anticoagulants and their analogs resulting in antiinflammation. Mounting observations also reveal antiinflammatory benefits resulting from anticoagulation (Fig. 2). The direct PAR blockade of the transmissions of blood coagulant mediators [for review, see ref. 17] and BK receptor antagonists show antiinflammation. Collectively, all of which further support such a coagulation-dependent inflammation phenomena.

4.1. Inhibition of Cytokine Production by Anticoagulation Approaches

4.1.1. Natural Anticoagulants

4.1.1.1. APC

Natural anticoagulant APC inactivates FVα and FVIIIa. In addition, APC exerts a profibrinolytic effect by inactivation of PAI-1 and inhibition of thrombin activated fibrinolytic inhibitor (TAFI) activation, which makes APC strong anti-thrombotic. As the result of the respective inhibitions on intrinsic tenase and prothrombinase for downregulating FXα and FIIa generation, APC thereby

| Table 1. Possible Antagonisms Against Blood Coagulation-Dependent Inflammation |
|---------------------------------|----------------|
| **Strategy**                  | **Selected Target**          |
| Anticoagulation                | Extrinsic coagulation       |
|                                | TF expression; TF/FVII activation; FVIIa; FXa; FIIa |
|                                | Intrinsic coagulation        |
|                                | FV; FVIII; FXIIa; KK          |
| PAR blockade                   | PAR-1/2/3/4                  |
| Contact system interface with complement | gC1qR/p33 |
| Complement inactivation        | C1; C3; C5; C3/C5 convertase |
| Prevention of complement activation | DAF/MCP/CD59/crry          |
| BK antagonism                  | B1 receptor; B2 receptor     |
inactivates the production of IL-1, -6, -8 or TNF-α [129]. APC is recognized one of the effective anti-inflammatory agents in clinical application. APC consistently reduces septic mortality and blocks DIC upon E. coli infection in either animal or human models [130-133]. In animal models, recombinant human soluble thrombomodulin (TM) prevents LPS-induced pulmonary vascular injury, inhibiting leukocyte activation [134, 135] and accumulation [136, 137]. In view of TM being a cofactor of FIIa, its facilitation of PC activation could further support anti-inflammatory significance in relation to APC anticoagulation.

4.1.1.2. Tissue Factor Pathway Inhibitor (TFPI)

With respect to its ability to directly inhibit FXa followed by a feedback inhibition on TF/FVIIa complex, natural anticoagulant TFPI suppresses coagulation-dependent IL-8 production [138] or VCAM-1 expression [139]. In VSMC, TFPI reduces the autocrine release of PDGF-BB, MCP-1 and MMP-2 in response to FVIIa and FXa [140]. Its coagulation-independent action includes the direct suppression in TNF-α, IL-6, and IL-8 production [141], reducing mortality from E. coli septic shock in baboons. TFPI also directly interferes with LPS reception [142]. The failure in human studies [143-145], however, warrants further research to clarify any clinical anti-inflammatory potential.

4.1.1.3. Antithrombin III (ATIII)

ATIII mediates pentasaccharide and heparin actions to inhibit FXa and FIIa, respectively. ATIII blocks FXa-induced IL-6, IL-8, MCP-1, ICAM/VCAM, and E-selectin expressions [146] in addition to arresting FIIa-induced (PAR-1-dependent) VEGF release [92] and MCP-1 expression [147]. Apart from inactivating NFκB [148], ATIII direct antiinflammatory action includes the suppression in INF-γ and ILs (e.g., 1, 2, 4, 6 & 8) production, which is mediated by enhanced PGI production and diminished inducible nitric oxide synthase (iNOS) [149]. However, the discrepancy exists concerning the survival rate being improved in baboons [150] but not in severe human sepsis treated with the high dose of ATIII [151]. Further research warrants verifying its anti-inflammatory potential.

4.1.2. Warfarin

Also known as Coumadin or Acenocumarol, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one is an inhibitor of the synthesis for vitamin K-dependent clotting factors [12]. It suppresses the reductases for vitamin K regeneration that is a cofactor for the posttranslational carboxylation of glutamates to form gamma-carboxyglutamates (Gla). The Gla domain is essential for phospholipid membrane anchoring and Ca²⁺ binding, which is present in the N-terminal of coagulation zymogens such as FII, FVII, FIX, FX, PC and PS [152]. Namely, warfarin generally depresses the functions of the clotting factors, thereby blocking global coagulation. Its antiinflammatory effect has been demonstrated in 1979 [153]. Oral warfarin significantly reduces IL-6 at day 15 [154]; further investigation warrants exploring broad anti-inflammatory benefits, if any.

4.1.3. FVIIa Inhibition

Recombinant nematode anticoagulant protein c2 diminishes coagulation-dependent IL-6 and IL-8 productions [51]. Active site-inhibited FVIIa depresses LPS-inducible plasma levels of TNF-α [144], IL-6 [155-157], and IL-8 [156, 157]. FVIIai abolishes VIIa signaling of Erk1/2 phosphorylation [83] in TF-expressing cells, while it suppresses sTF-induced inflammation in vivo model [48]. A small molecule BCX-3607 (TF/FVIIa inhibitor) also decreases IL-6 level in an endotoxemia mouse model [158].
4.1.4. FXa Inhibition

L-MWH, enoxaparin, or DX9065a suppresses P-selectin, TF-α, IL-6 [159], or MCP-1 [160] expression, resulting in depressed platelet activation [161] and leukocyte adhesion to EC [162]. A direct inhibitor (ZK-807834) blocks FXa signaling of eliciting IL-6 [87]. The anti-inflammatory effects of a newer anticoagulant Rivaroxaban, however, remain largely unknown.

4.1.5. FIIa Inhibition

Heparin shows a variety of inflammatory potentials [for review, see ref. 163]. Heparin-bonded circuit prevents the increases in IL-6 and IL-8 in CPB patients without any effect on P-selectin [164], while heparin bolus reduces neutrophil activation without affecting platelet aggregation [165]. Heparin and delteparin downregulate PAR-1 cleavage [166], blocking PAR-1-mediated VEGF release in response to FIIa [92].

Direct FIIa inhibitor (hirudin) binds to FIIa active site and prevents PAR-1 from cleavage [166], thereby diminishing FIIa signaling in ICAM/VCAM expression [167] and elicitation of VEGF [63, 92], IL-6 [168], IL-8 [58], or MCP-1 [58]. Hirudin suppresses sTFI-219-induced inflammation [46]. A hirudin analog (lepirudin) alleviates LPS-induced platelet activation [169], and an active site inhibitor (melagatran) diminishes P-selectin expression [166]. Whether dabigatran could show any inflammatory effect warrants further study.

4.2. Antiinflammation by Blockade of Contact System

Consistent with the fact of FXIIa and KK being proinflammatory (section 3.1.6.), PA (urokinase) readily downregulates contact system with the consequence of lowering BK production and complement inactivation, accounting for its prevention of inflammation [128].

4.2.1. FXIIa Inhibition

C-1 inhibitor, a protease inhibitor of the serpin family, downregulates contact coagulation by inactivating FXIIa, showing antiinflammation [102, 170]. ATIII-bound heparin and heparin sulfate inhibit FXII activation [171]. Ectoin is a potent inhibitor for FXIIa [172].

4.2.2. KK Inhibition

Antiinflammatory contribution of KK inhibition is essentially mediated by blocking complement activation. C-1 inhibitor inactivates KK [102, 170] together with its ability to directly suppress complement C-1 [173], showing diverse anti-inflammatory effects.

A recombinant small protein Ecallantide (DX88) [174], based on the first Kunitz domain of human TFPI, is a potent and specific inhibitor of plasma KK; DX88 reverses the increased vascular permeability in C-1 inhibitor deficient mice. Ectoin [172] and Aprotinin [175] also directly inhibit KK and suppress BK release. However, anti-inflammatory effects by such inhibitors have not been reported thus far.

4.2.3. BK Inhibition

BK antagonists have already shown antiinflammatory action by improving survival of septic animals several decades ago. A wide variety of BK and B-1/B-2 receptor antagonists are currently available for clinical applications for antiinflammation [for review, see ref. 176]. It, however, remains to be determined if these antagonisms specifically block coagulation-dependent inflammation.

5. REMARKS

Coagulation-dependent inflammation is revealed by which coagulant mediators are proinflammatory (Fig. 1) and anticoagulation is of anti-inflammation (Fig. 2). Furthermore, coagulation-mediated platelet and complement activations readily present an array of inflammation (Fig. 3). Hypercoagulation per se elicits elevated generation of FVIIa, FXa, FIIa, FXIIa, KK, and BK as well as fibrin overproduction, all of which in turn initiate cellular activation and signaling for inflammation (Fig. 1). Both extrinsic and intrinsic pathways in fact play diverging as well as converging roles in proinflammation. Such vicious cycle linking blood coagulation to inflammation mutually refuels each other, ensuring hypercoagulability as well as hyper-inflammatory (Fig. 2). In these regards, blood coagulation could become a target for antiinflammation.

With respect to its thrombogenic as well as proinflammatory natures, it is not surprising that hypercoagulability triggers diverse cardiovascular complications. Namely, hypercoagulability extends one arm for thrombotic consequence and the other for inflammatory events. A paradigm: circuit (Fig. 3) integrates the coagulation-inflammation cycle to thrombosis [for review, see ref. 127] that is closely related to inflammation [for review, see ref. 126]. Accordingly, any interruption of the circuit could be of cardioprotection. Table 1 summarizes possible approaches to antiinflammation in relation to its blood coagulation-dependence. Apart from anticoagulants described in the above section 4, antagonism against complement components, PARs, cytokine reception, or gC1qR/p3 could be expected to achieve antiaggregation, antiinflammation, antithrombosis, and cardioprotection. Even nutrient such as antioxidant curcumin suppressing TF production, all of which in turn initiate cellular activation accounting for thrombotic consequence and the other for inflammatory events. A paradigm: circuit (Fig. 2) integrates the coagulation-inflammation-thrombosis circuit, showing antiinflammation [for review, see ref. 176]. It, however, remains to be determined if these antagonisms specifically block coagulation-dependent inflammation.

Anticoagulation not only blocks thrombogenesis, but also arrests the coagulation-inflammation cycle (Figs. 2, 3). The biopharmaceutical applications of anticoagulant development could be several-fold. (1) Anticoagulation certainly confers intervention remedy by offsetting the induced extrinsic hypercoagulability resulting from inflammation, vascular injury, or circulating TF microparticles. (2) Anticoagulation could provide broad spectrum of anti-inflammatory relevance regarding the operational coagulation-inflammation vicious cycle. (3) In view of hypercoagulability driving the circuit linking thrombosis and inflammation (Fig. 3), anticoagulants block the coagulation-inflammation cycle to interrupt the coagulation-inflammation-thrombosis circuit, showing cardioprotection. The ability of PAR1 and 2 to induce TF [177] reveals not only coagulation-dependent inflammation but also diverging and converging roles of TF in refueling the cycle [17]. It remains challenging that selective upstream antagonism (i.e., inhibition on TF-dependent FVII activation) arrests only the extrinsic hypercoagulability to prevent thrombotic complication instead of blocking the
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constitutive intrinsic coagulation for hemostasis, which would effectively suppress inflammation while avoiding bleeding episode.

**ABBREVIATIONS**

- AP-1 = Activator protein-1
- APC = Activated protein C
- AT II = Angiotensin-II
- AT III = Antithrombin III
- BK = Bradykinin
- COX-2 = Cyclooxygenase-2
- CRP = C-reactive protein
- Egr-1 = Early growth response-1
- ET-1 = Endothelin-1
- FBG = Fibrinogen
- FIa = Thrombin
- FVIIa = Activated factor VII
- FVIIai = Active-site inhibited FVIIa
- FXa = Activated factor X
- HK = High molecular weight kininogen
- ICAM = Intracellular adhesion molecule
- IL = Interleukin
- IRF3 = Interferon response factor 3
- JAK = Janus-activated kinase
- KK = Kallikrein
- LDL = Low density lipoprotein
- LMWH = Low-molecular-weight-heparin
- Lp(a) = Lipoprotein (a)
- LPS = Lipopolysaccharide; bacterial endotoxin
- MAPK = Mitogenic activating protein kinase
- MCP = Monocyte chemotactic protein
- MMP = Matrix metalloproteinase
- NF-κB = Nuclear factor –κappa B
- NOS = Nitric oxide synthase
- OxLDL = Oxidized LDL
- PA = Plasminogen activator
- PAF = Platelet activating factor
- PAI-1 = Plasminogen activator inhibitor-1
- PAR = Protease activated receptor
- PC = Protein C
- PDGF = Platelet derived growth factor
- PF 1+2 = Prothrombin fragments 1+2
- PGE2 = Prostaglandin E2

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**Fig. (3).** The paradigm: coagulation-inflammation-thrombosis circuit, implication on cardiovascular events. Hypercoagulability with elevated coagulant mediators, fibrin overproduction, complement activation, and platelet activation results in inflammation (elevated production of cytokines, adhesion molecules, growth factors, and other mediators) as well as thrombosis (thrombus, platelet aggregation, fibrin deposit). A circuit links the coagulation-inflammation cycle and inflammation-thrombosis association. Inflammation and thrombosis are two risk factors for cardiovascular events. Accordingly, any interruption of the cycle and/or any part of the circuit could exhibit cardioprotection benefits.
PKC = Protein kinase C
PKK = Prekallikrein
PN-2/AbPP = Protease nexin-2 amyloid beta protein precursor
PTK = Protein tyrosine kinase
RA = Rheumatoid arthritis
ROS = Reactive oxygen species
SMC = Smooth muscle cell
TAP = Tick anticoagulant peptide
TAT = Thrombin-antithrombin complex
TF = Tissue factor
TFPI = TF pathway inhibitor
TGF = Transforming growth factor
TLR = Toll-like receptors
TM = Thrombomodulin
TNF-α = Tissue necrosis factor-alpha
TT = Thrombin time

REFERENCES


Coagulation-Dependent Inflammation


