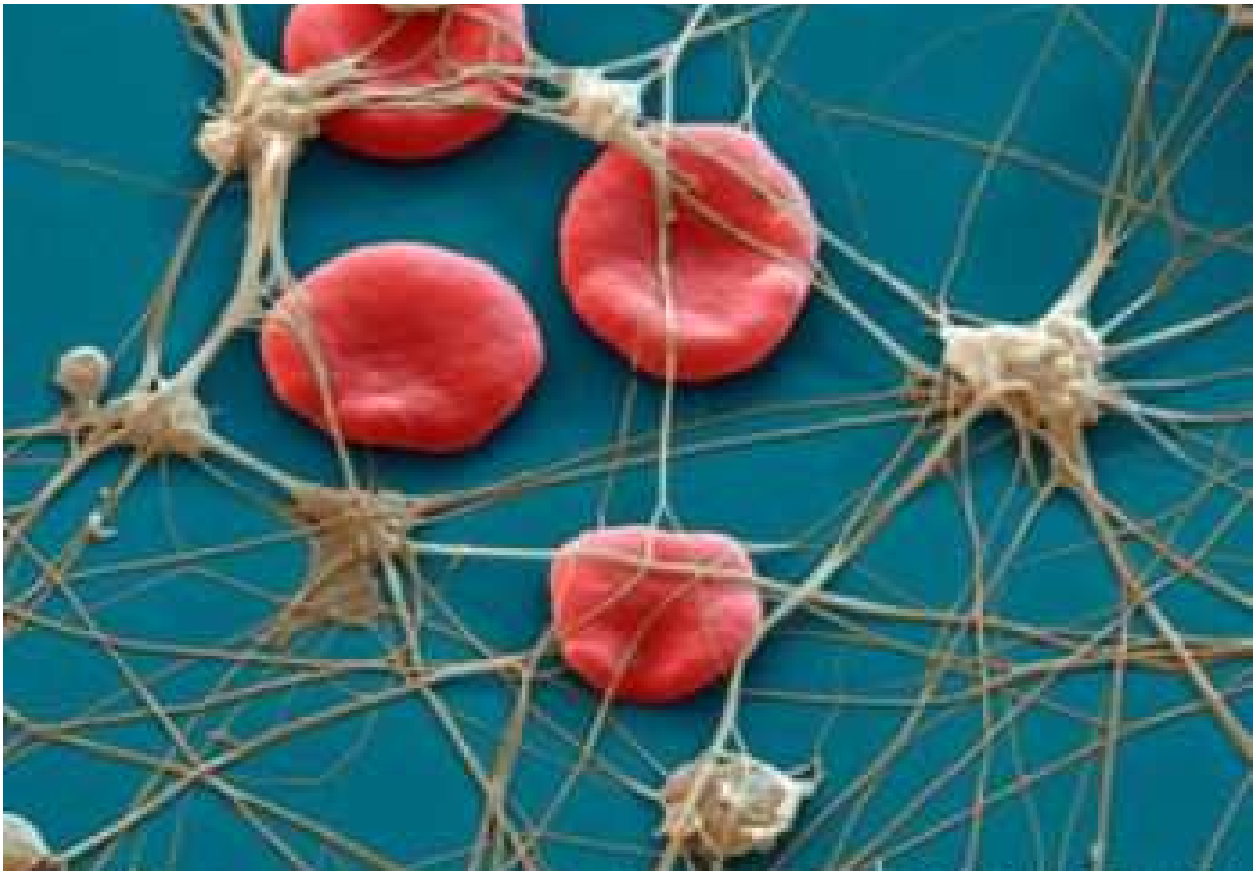
The coagulation system and its role in bacterial infections



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About the cover

Scanning electron micrograph of a fibrin clot with erythrocytes trapped in the fibrin network. Bacteria can also become trapped in the fibrin clot that is a generated as result of the activation of the coagulation system. This report focuses on the role of the coagulation cascade during bacterial infections. (Taken from Science Photo Library).

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The coagulation system

The coagulation system is induced upon damage of the blood vessel wall and its major function is to induce hemostasis. Coagulation factors circulate in the blood as inactive proteases which can sequentially activate each other and induce the coagulation cascade. This results in the formation of fibrin which seals of the wound and stops bleeding. In addition, the coagulation system is important in host responses to bacterial infection and the local formation of fibrin prevents spread of invading pathogens. However, invasive bacteria are capable of evading this response by stimulating or suppressing the activity of host proteases. This thesis describes some examples of bacterial interactions with different stages of the coagulation system and the results for bacterial dissemination. In addition, recent findings on the role of coagulation in the inflammatory response and the activation of the complement system are discussed.

Initiation of the coagulation cascade occurs when tissue factor (TF) is exposed after vessel wall injury. TF is a 45-kDa transmembrane protein that is constitutively expressed by subendothelial cells and fibroblasts and is normally shielded from contact with blood components[1]. However, damage to the endothelium exposes TF, which then binds and activates factor VII (FVII) (Figure 1). The generated FVIIa/TF complex activates the circulating factors X (FX) and IX (FIX). Activated FX (FXa) and FIX (FIXa) provide positive feedback by activating FVII that is bound to TF. FXa and FIXa remain associated with the TF-expressing cell or might dissociate and bind to the membrane of activated platelets[2]. In turn, FXa associates with activated factor V (FVa) and forms the prothrombinase complex which converts prothrombin to thrombin. The generated thrombin induces a positive feedback loop by activation of circulating FV and FVIII, which are important cofactors to TF and FIX, respectively[2]. The major function of thrombin is to convert fibrinogen to fibrin[3]. Fibrin is composed of six polypeptides chains, two α-, β-, and γ- chains, linked by disulphide bonds and becomes activated when thrombin cleaves the α- and β-chains[4]. The fibrin molecules are cross-linked by FXIIIa activity.

Activation of the coagulation cascade via TF is referred to as the extrinsic pathway. This pathway is induced as response to damage to the vessel wall. However, the coagulation system can also be initiated through activation of the contact system (intrinsic pathway). Activation of the contact system results in the formation of fibrin via subsequent activation of FX. Furthermore, it leads to the release of inflammatory mediators and antimicrobial peptides via activation of the kallikrein-kinin system. The intrinsic pathway is initiated by activation of factor (FXII) (Figure 1). FXII can autoactivate itself by cleavage, but this is not very efficient. Although the main mechanism of activation is unknown, FXII can bind to negatively charged surfaces, including polyphosphates, nucleic acids, and collagen. Binding to these surfaces induces a conformational change and makes the protein more susceptible to cleavage[5]. Activated FXII (FXIIa) converts factor XI (FXI) to its active proteolytic form. Further cleavage of the C-terminal of FXII release the 30 kDa fragment FXIIf, which stimulates plasma kallikrein (PK). Activated PK can convert surface-bound FXII more efficiently, resulting in amplification of the number of activated molecules[5]. In addition, activated PK cleaves the circulating high-molecular-weight kininogen (HK) and releases the peptide bradykinin (BK). BK is a proinflammatory peptide which induces vascular leakage through interaction with receptors on endothelial cells. This facilitates the recruitment of innate immune cells, including neutrophils and monocytes. Activated FXI (FXIa) stimulates FVIII, which in turn converts IX to its active form and induces the production of thrombin and fibrin via FX[6]. Moreover, FXIa stimulates the extrinsic pathway of coagulation via activation of FVII. Both pathway converge when FX is activated.

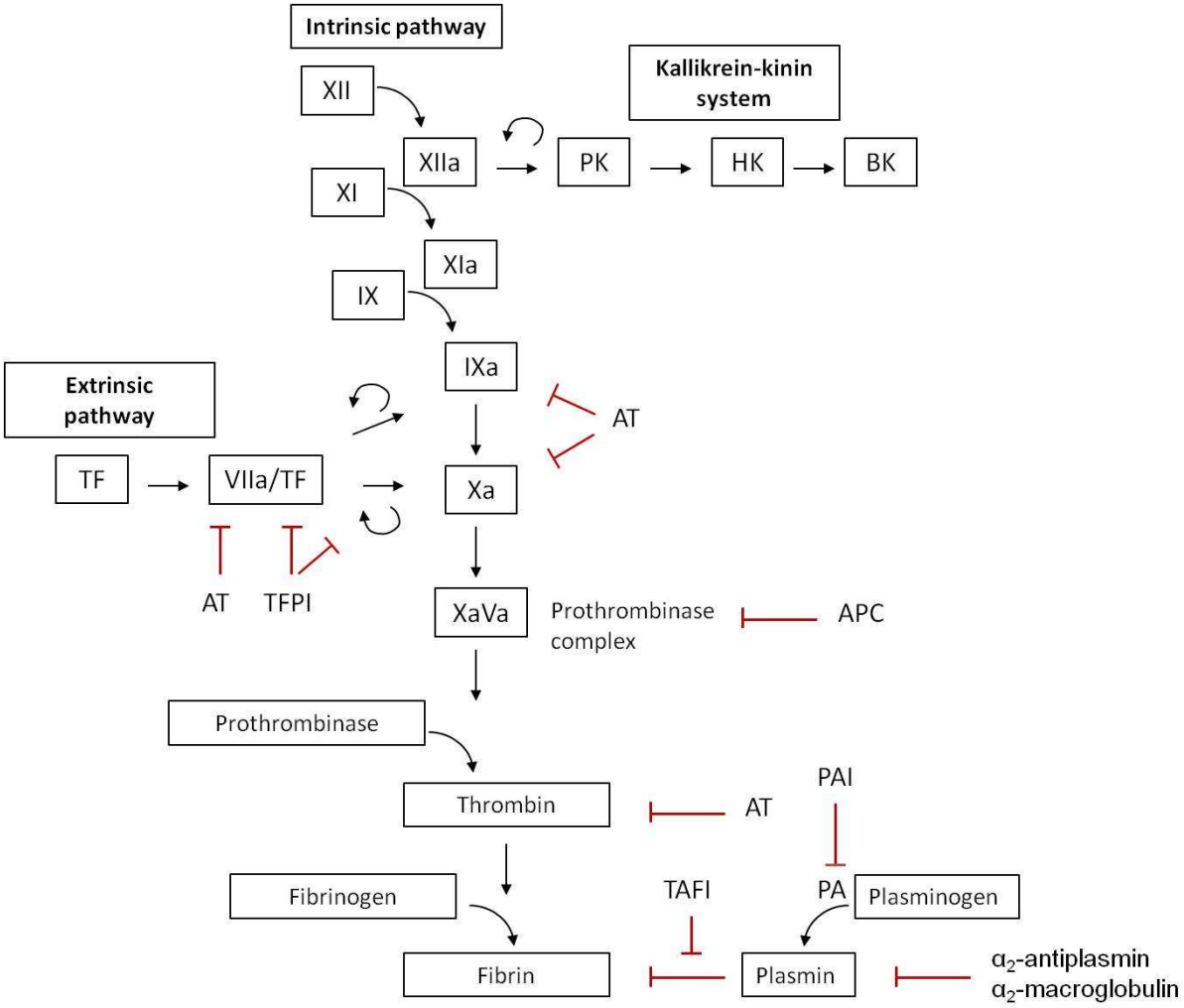

Figure 1. Scheme of the coagulation system. The coagulation system can be activated through the intrinsic and extrinsic pathway and subsequently results in the formation of fibrin at the site of injury. The activation of the kallikrein-kinin system, followed by the release of kinins, results in vascular permeability. The coagulation system is negatively regulated by tissue factor protein inhibitor (TFPI), activated protein C (APC), antithrombin (AT) and plasmin. In turn, the formation of plasmin in catalyzed by plasmin activators (PA) and can be inhibited by plasmin activator inhibitors (PAI). Thrombin activated fibrinolytic inhibitor (TAFI) prevents the breakdown of fibrin clots by plasmin.

Regulation of the coagulation system

The coagulation process is tightly regulated at different stages of the cascade to slow the formation of blood clots and prevent systemic blood coagulation. Coagulation inhibitors and the fibrinolytic system are important in maintaining this hemostatic balance. The three major anticoagulants are tissue factor pathway inhibitor (TFPI), antithrombin (AT) and activated protein C (APC). TFPI is a serine protease that is present in plasma and on vascular cells and can bind and inhibits FXa. The TFPI-FXa complex clusters on the membrane surface, due to the ability of FXa to bind negatively charged surfaces. As a result, the complex can also inhibit the surface-associated FVIIa[8]. This suppresses the activity of the TF-FVIIa complex and inhibits further activation of FIX And FX[3]. AT is produced by the liver and inhibits several coagulation factors, including thrombin, FVIIa, FIXa and FXa[3]. Protein C is an inactive plasma serine protease which can be activated by the thrombin/thrombomodulin complex on vascular endothelial surfaces. The thrombin molecules that are produced during the coagulation process can bind thrombomodulin. The formed complex can cleave and

activate protein C when it is bound to the endothelial protein C receptor (EPCR). APC dissociates from the EPCR and forms a complex with cofactor protein S. The protein C-protein S complex negatively regulates the coagulation process by inactivation of FVa and FVIIIa[8]. The activity of FIXa and FXa is severely impaired without these cofactors, resulting in almost complete blockage of the coagulation cascade. The thrombin/thrombomodulin complex is efficiently inhibited by AT[8].

The function of the fibrinolytic system is to degrade existing fibrin clots. The serine protease plasmin is a major protease of fibrinolytic system. Plasmin is produced by the liver and circulates in the blood in high concentrations (180 μg/ml) as inactive proenzyme plasminogen[9]. Plasminogen is a 92-kDa glycoprotein and consists of an N-terminal preactivation peptide, five Kringle domains and a protease domain. Plasmin has a broad range of substrates, including fibrin and laminin and degrades fibrin clots as well as extracellular matrix and basal membranes. Plasminogen is activated by tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). The binding of plasminogen to these receptors is mediated by lysine-binding Kringle domains. Binding to receptors, fibrin clots or bacterial surfaces immobilizes plasmin and makes it less susceptible to inactivation by plasmin inhibitors, such as α_2-antiplasmin and α_2-macroglobulin[10]. t-PA and u-PA are expressed by leukocytes and expression is up-regulated during infection. tPA and uPA are activated by PK. Release of tPA from endothelial cells is increased by BK activity[5]. While tPA mainly activates the fibrinolytic pathway, uPA has low affinity for fibrin and can bind to its receptor (uPAR) on leukocytes, thereby stimulating cellular adhesion and migration[11]. The fibrinolytic pathway is regulated by plasmin inhibitors and plasminogen activator inhibitors (PAI) 1-3[9]. Circulating PAI is often bound to vitronectin. PAI 1-3 can inhibit both t-PA and u-PA by complex formation and distorts the active site of these proteases[3]. However, PAI-1 can be inactivated by FXII[5]. The thrombin activated fibrinolytic inhibitor (TAFI) inhibits fibrinolysis by removing C-terminal lysine residues from fibrin. This alters the binding sites for plasmin[5].

Proinflammatory coagulation factors

It has been shown that the contact system can modulate the inflammatory response and is involved in the local host response against pathogens. Monocytes and neutrophils are directly activated by components of the contact system, e.g. PK and FXIIa. This results in aggregation and degranulation of neutrophils and interleukin (IL)-1 production by monocytes[5]. An important link between inflammation and the coagulation system are the protease-activated receptors (PARs). PARs are expressed by endothelial cells, mononuclear cells, platelets, fibroblasts and smooth muscle cells. PAR 1-4 belong to the family of G-protein-coupled receptors. Proteolytic cleavage of PARs by coagulation factors results in exposure of a neoamino terminus. This results in autoactivation and initiates signaling. Thrombin is the ligand of PARs 1, 3 and 4, whereas PAR-2 functions as receptor for the TF-FVIIa complex and FXa. PAR-3 is an accessory receptor for PAR-1 and PAR-4. In addition, PAR-1 can be activated by FXa and APC. In contrast to activation of PAR-1 by thrombin, cleavage by APC has contradictory effects, including desensitization of PAR1 and downregulation of the inflammatory response.

Activation of the kallikrein-kinin system results in the release of vasoactive kinins. Kinins bind to the two human kinin receptors B1R and B2R. These receptors belong to the family of G-protein coupled receptors. Signaling promotes neutrophil migration via activation of phospholipase C, mobilization of intracellular calcium by inositol-1,4,5-triphosphate (IP3) and activation of protein kinase C[12]. B2R is constitutively expressed on most cell types, whereas expression of B1R is up-regulated upon stimulation by pro-inflammatory agents, including interleukin 1β and endotoxin. Binding of kinins to neutrophils promotes migration, whereas binding to endothelial cells releases nitric oxide and prostacyclin and induces vasodilation and smooth muscle cell relaxation[12].

Host defense peptides

Recently, it has been shown that cleavage of coagulation factors might result in the generation of host defense peptides. In contrast to intact proteins, which show no activity, proteolysis of human prothrombin and thrombin by neutrophil elastase generates antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa* for several hours *in vitro*[13,14]. Elastase is normally present during blood coagulation and inflammation and is secreted by neutrophils. Papareddy *et al.* showed that thrombin-derived C-terminal peptides (TCP) were produced when fibrin clots or human plasma was incubated with neutrophil elastase and induce lysis of microbial membranes[13]. Furthermore, TCPs can be detected in human wounds. In addition, peptides derived from FIX, FX, plasminogen and protein C showed antimicrobioal activity against *E. coli and P. aeruginosa*[13,15]. Moreover, these peptides have immunomodulatory activity. Injection of mice with LPS in combination with C-terminal peptides derived from thrombin, FIX, FX or plasminogen resulted in reduced pro-inflammatory cytokine production (IL-6, IFN-γ, TNF-α and MCP-1), whereas the production of anti-inflammatory IL-10 was increased[15,16].

In addition to the thrombin C-terminus, C-terminal TFPI peptide sequences show antimicrobial activity against Gram-negative (*E. coli*, *P. aeruginosa*) as well as Gram positive (*B. subtilis*, *S. aureus*) bacteria and fungi. TFPI is cleaved by different proteinases, including thrombin and plasmin. This generates various C-terminal truncated forms of TFPI, which can be found in human plasma. The C-terminal peptides induce enhanced binding of C1q and C3a to the bacterial surface[16].

Bacterial interactions with host coagulation factors

The coagulation system also plays a role in elimination of pathogenic bacteria. As a result of the formation of fibrin clots, bacteria become immobilized inside the fibrin network and spread into the surrounding tissues can be prevented. It has been shown that mice deficient in FV or fibrinogen show increased mortality after infection with group A streptococci (GAS), due to decreased fibrin production[17]. This indicates that the coagulation system is important in the host defence against GAS. However, many bacterial pathogens have the capacity to interact with coagulation factors of the host and thereby induce disruption of the fibrin clot (Figure 2 and Table 1). In addition, various pathogenic bacteria express receptors to interfere with other stages of the coagulation cascade. The contact pathway can be activated at the bacterial surface through interaction with bacterial surface organelles, such as curli organelles or fimbriae of *Escherichia coli* (*E. Coli*) and *Salmonella spp.*, respectively[18,19]. Activation of the contact system results in the generation of kinins, which might facilitate bacterial spread into the surrounding tissue by enhancing the vascular permeability.

Release of kinins

Several streptococcal serotypes, including *Streptococcus pyogenes*, bind kininogens by their M proteins[3]. When kininogens are bound to the bacterial surface, they become more susceptible to cleavage and activation by host proteases. Kininogen-binding receptors are viewed as virulence factors, since the release of vasoactive components might be beneficial to bacterial dissemination.

The cysteine proteinases staphopain A (ScpA) and staphopain B (SspB) from *Staphylococcus aureus* and streptopain (SpeB) from *Streptococcus pyogenes* act directly on HK in human plasma to release BK[20,21]. In addition to direct activation of kininogens, Aeromonas sobria serine proteinase (ASP) from the gasteroenteritis causative agent *A. sobria* is capable of releasing BK from HK through proteolytic activation of prekallikrein. The induction of vascular leakage has been confirmed in a guinea pig infection model of *A. sobria*[22].

Commensal bacteria also initiate the contact system and generate BK. The Gram negative *Bacteriodes fragilis* and *Bacteroides thetaiotaomicron* can bind and activate HK and degrade fibrinogen, thereby increasing the clotting time *in vitro*[23]. *Bacteriodes* species are commensal bacteria residing in the gut, but can cause opportunistic infections outside the gut and are often the cause of post-operative complications[23]. Both serotypes can activate PK and bind and cleave HK, thereby releasing BK. Furthermore, both serotypes are able to recruit and exploit FXI and FXII, thus initiating the intrinsic coagulation pathway[23]. However, *B. fragilis* interacts with the β–chain of fibrinogen by the B. fragilis fibrinogen-binding protein (BF-FBP) and induces fibrinogen hydrolysis[4]. Depletion of fibrinogen prevents the formation of a fibrin network. The ability to modulate clot formation might increase the virulence of these opportunistic bacteria. However, there are no studies of *in vivo* BF-FBP expression.

Exploitation of the fibrinolytic system

Many bacteria can bind circulating plasminogen to their surface where it can be converted to active plasmin and subsequently induces fibrinolysis. Activation of the fibrinolytic cascade results in degradation of the extracellular matrix, basal membrane and host tissues and facilitates bacterial spread. Recruitment of plasminogen is important for the dissemination of *Borrelia burgdorferi*, since this bacterium lacks surface protease activities[24].

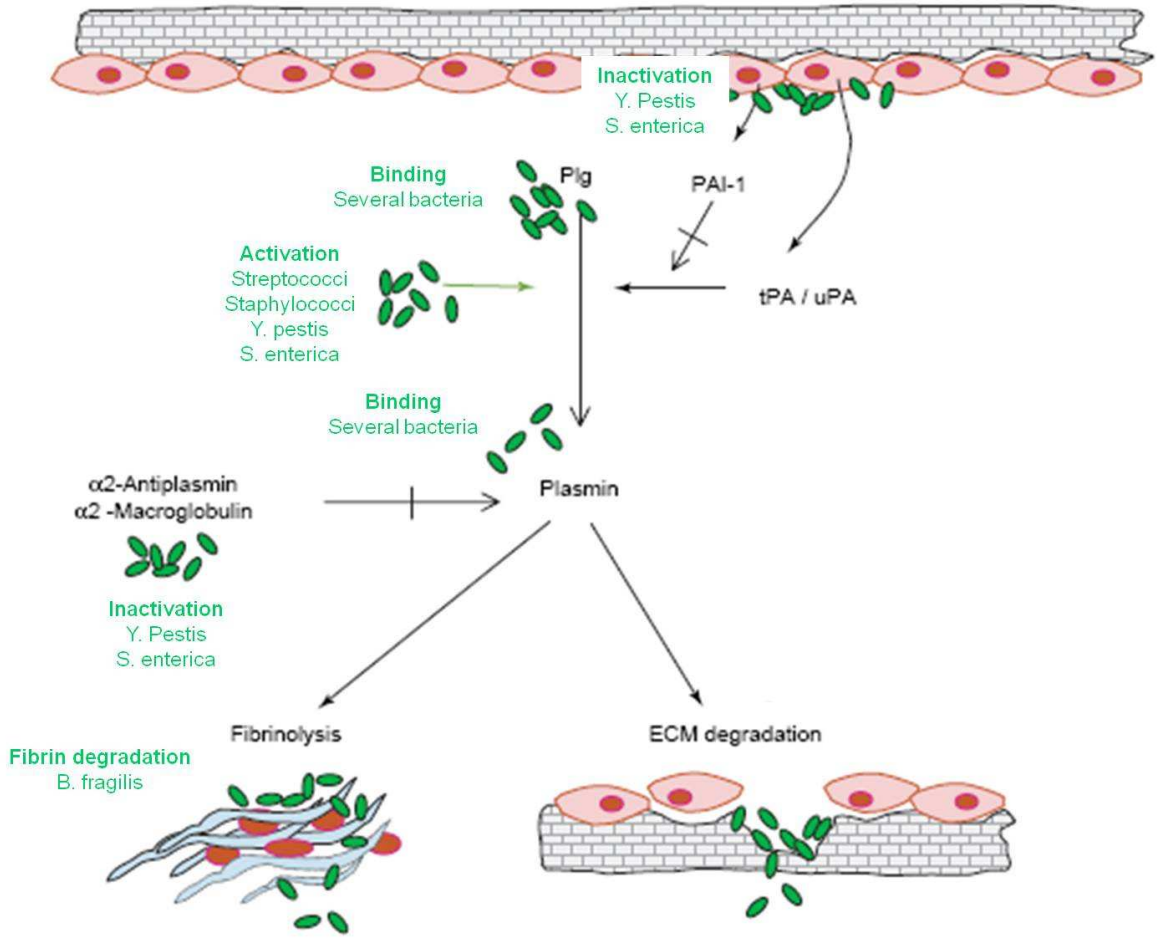

Figure 2. Bacterial interactions with the host fibrinolytic system and subsequent induction of fibrin and extracellular matrix degradation. Plasmin is the major protease of the fibrinolytic system and its ability to degrade components of the extracellular matrix is exploited by various pathogenic bacteria. The activation of plasminogen is regulated by plasminogen activators (tPA and uPA), plasminogen activator inhibitors (PAI) and plasmin inhibitors, including α_2-antiplasmin and α_2-macroglobulin. These factors can also be inhibited by certain bacteria (adapted from Lähteenmäki *et al.*, 2005[25]).

B. Burgdorferi is transmitted by infected ticks and causes Lyme disease. These bacteria express various structures to bind plasminogen to the bacterial surface, which can be activated by host uPA. The outer surface protein C (OspC) is transiently up-regulated after infection of the human host, while OspA is mainly expressed within thicks. Both proteins can bind plasminogen and the surface-bound active plasmin is used to invade host tissues[26]. Simultaneously, *B. burgdorferi* induces overexpression of uPAR in monocytes, which enhances the local plasminogen activation[26]. *B. burgdorferi* also expresses the plasminogen-binding Erp proteins (ErpA, ErpC, ErpP) through all stages of mammalian infection and establishes plasmin activity during the chronic phase of infection[24].

Other bacteria, including *Haemophilus influenza*, also depend on host uPa to convert surface-associated plasminogen into plasmin. *H. influenza* is associated with upper and lower respiratory tract infections. The N-terminal region of the 16 kDa *Haemophilus influenza* surface protein E (PE) binds plasminogen, but is incapable of activating it[27].

Group A, C and G streptococci produce the plasminogen activator streptokinase (SK). The streptokinase on the surface of the bacterium associates with plasminogen and induces conformational changes thereby converting plasminogen into its active form plasmin[9]. This conformational change also protects against inactivation by α_2-antiplasmin. Streptococcal streptokinase is highly specific for human plasminogen[28]. Another plasmin activator with a similar function is produced by *Staphylococcus aureus*, named staphylokinase (SAK). SAK

requires fibrin as co-factor and the formed complex protects against degradation by α_2-antiplasmin[3]. In addition to degradation of the fibrin network and extracellular matrix components, the surface-associated plasmin can degrade the opsonins C3b and IgG and plays a role in immune avoidance[29]. This has also been shown for *Bacillus anthracis*, the causative agent of anthrax, after activation of plasminogen by host uPA[30]. *B. anthracis* binds plasminogen by α-enolase. Several other Gram positive and Gram negative bacteria, including Group A streptococci and *B. burgdorferi*, also bind and activate plasminogen via enolases[10, 31, 32]. Enolases are glycolytic enzymes that are located intracellularly and on the surface of eukaryotic and prokaryotic cells. The surface-expressed enolases can bind plasminogen, although the affinity of bacterial enolases for plasminogen is higher compared to eukaryotic enolases[31]. The surface-bound plasminogen can be activated by host tPA.

In contrast to plasminogen activation, Sijbrandi *et al.* suggested that certain bacteria might bind plasminogen and shield it from plasminogen activators[33]. This would prevent fibrin degradation and might avoid detection of the invading pathogen by migrating leukocytes. An example is the plasminogen binding protein (Pbp) of *B. fragilis*, which is present on the bacterial surface. However, the surface-assoaciated plasminogen is not activated[33]. Pbp-like proteins were found in clinical isolates of *B. thetaiotaomicron, B. vulgatus*, *B. ovatus* and *P. distasonis*[33].

Suppression of coagulation inhibitors

Other bacterial species inactivate the protease inhibitors that regulate plasmin activation and thereby induce uncontrolled fibrinolysis. The surface protease PgtE of *Salmonella enterica* serovar Typhimurium interferes with multiple components of the fibrinolytic system. It converts plasminogen to plasmin and is also able to bind and inhibit α_2-antiplasmin[11]. Furthermore, PgtE of *S. enterica* suppresses PAI-1 activity by cleavage of the reactive center loop, which impairs interaction with target proteins[11]. PgtE can cleave soluble PAI-1 and vitronectin-associated PAI-1, which is often found in circulation[11]. Similar results are shown for *Yersinia pestis* and the β-barrel outer membrane protease Pla. Pla and PgtE belong to the Pla subfamily of omptins[11]. Pla converts plasminogen to plasmin by mimicking the action of tPA and uPA. Furthermore, Pla inactivates α_2-antiplasmin and PAI-1. Interestingly, *Y. pestis* spreads mainly extracellularly, whereas *S. enterica* spreads within phagocytic cells, indicating that omptins are important virulence proteins for both invasion mechanisms[11]. In contrast, other bacterial species have been shown to suppress the fibrinolytic cascade and thereby promote fibrin deposition. Patients with *Helicobacter pylori* infection show enhanced levels of PAI-2. *H. pylori* produces neutrophil activating protein (HP-NAP) that is able to activate NADPH-oxidase in neutrophils. However, it has been shown that HP-NAP also stimulates PAI-2 and TF production by mononuclear cells independent of NADPH-oxidase and without enhanced expression of uPA on these cells[34]. The uncontrolled fibrin formation might be beneficial for the development chronic gastritis and impairs the migration of phagocytes towards the site of infection.

Bacterial clustering

Recently, Kastrup *et al.* showed that the coagulation cascade can be initiated by clusters of *Bacillus cereus* and *Bacillus anthracis* in human blood plasma. The number of *B. cereus* bacteria that induces rapid coagulation is much lower in a cluster (~$4x10^3$ CFU), compared to the number of bacteria in solution (~10^8 CFU)[35]. However, not all types of bacterial clusters induce coagulation. Stains of *E. coli* lacking curli fibers are incapable of initiating coagulation. Clusters of this strain also lacked the ability induce coagulation, indicating that clustering itself is not sufficient to initiate the coagulation process. Kastrup *et al.* suggests that the rapid initiation of coagulation by bacterial clusters (within 3 minutes) is not induced by

quorum sensing, since mutant bacterial strains of *B. anthracis* with reduced quorum sensing abilities were also capable of inducing rapid coagulation of blood plasma. Nevertheless, the bacterial clusters might generate high local concentrations of activated coagulation factors, thereby exceeding a threshold and inducing coagulation by 'quorum acting'[35,35].

Table 1. Bacterial proteinases that interfere with components of the coagulation cascade.

Bacteria	Bacterial protein	Function
Aeromonas sobria	ASP	PK activation
Bacteriodes fragilis	BF-FBP Pbp	Fibrin hydrolysis Plasminogen binding
Borrelia burgdorferi	OspC, Erp proteins	Plasminogen binding
Group A streptococci	SK Enolases	Plasminogen activation Plasminogen binding
Haemophilus influenza	PE	Plasminogen binding
Helicobacter pylori	HP-NAP	PAI-2 upregulation
Salmonella typhimurium	PgtE	Plasminogen activation α_2-antiplasmin inhibition PAI-1 inactivation
Staphylococcus aureus	ScpA, SspB SAK	Release of BK from HK Plasminogen activation
Streptococcus pyogenes	M protein SpeB	Kininogen binding Release of BK from HK
Yersinia pestis	Pla	Plasminogen activaton

Interactions with the complement system

The coagulation pathways and complement system are often described as separate cascades. However, both system descent from a common ancestral pathway and show similarities. Comparable to the coagulation system, the complement system consists of a proteolytic cascade of serine proteases and is part of the innate defense system against invading pathogens. Activation of complement factors results in rapid recognition and opsonization of pathogens, recruitment of innate immune cells and elimination of target cells. It has been shown that the coagulation and complement systems form a complex interactive network during inflammation in which coagulation factors can initiate the pathways of complement activation and vice versa.

The complement system can be activated through three major pathways: the classical pathway, the alternative pathway and the lectin pathway[36]. All pathways subsequently induce the formation of the lytic membrane attack complex (MAC). MAC can insert in the bacterial membrane and induces lysis of the target cell.

The classical pathway
The classical pathways is induced by binding of antibodies (Ab) to specific pathogens. Binding of the pathogen induces a conformational change in the Fc-region of the Ab, which allows binding of circulating C1q. C1q can subsequently activate C1r, which leads to cleavage of C1s. In turn, C1s converts C4 into C4a and C4b. C4b can bind and convert C2 into C2a and C2b on the bacterial surface. C4b forms the surface-bound C3 convertase complex with C2a, while C2b diffuses. The C3 convertase cleaves C3 into C3a and C3b. C3a functions as anaphylatoxin and can recruit innate immune cells. C3b forms a complex with C4b2a (C4b2a3b), which initiates the last phase of the complement cascade and results in the formation of the lytic membrane attack complex (MAC). Furthermore, C3b can also opsonize pathogens and thereby promote phagocytosis.

The alternative pathway
Activation of the alternative pathway is triggered by danger signals of infected cells, but also by polysaccharides or damaged tissue. Subsequent hydrolysis of C3 results in generation of C3(H_2O), which resembles C3b. C3(H_2O) associates with factor B and can be cleaved by the circulating protease factor D in order to produce the fragment Ba. This is followed by the formation of the C3 convertase C3(H_2O)Bb. This complex is stabilized by binding of the protein properdin. C3(H_2O)Bb converts C3 into C3a and C3b. Together with factor B and D, C3b can generate new C3 convertases and thereby amplifies the number of activated molecules. This amplification loop is also important for the classical and lectin pathway. Binding of C3b to the C3 convertase generates the C5 convertase C3(H_2O)BbP3b, which in turn can initiate the terminal cascade and results in the formation of MAC.

The lectin pathway
The lectin pathway is activated when mannose binding lectin (MBL) binds mannose-containing surface proteins on the surface of pathogenic bacteria. MBL forms a complex with the (MBL associated) serine proteases MASP-1 and MASP-2, which resembles the structure of the C1q complex with C1s and C1r of the classical pathway. MASP-2 catalyzes the cleavage of C2 and C4 upon activation and stimulates the formation of the C3 convertase C4b2a. MASP-1 is also capable of C2 and C3 cleavage, although less efficient. In contrast, MASP-3 does not participate in complement activation, but can inhibit MASP-2 function. The formed C3b (from C3) associates with the complex to form a C5 convertase (C4b2a3b). The

lectin pathway can also be activated via binding of MASPs to ficolins. Ficolins are collagen-like structures that bind to sugars presented on microorganisms and dying host cells. There are three different ficolins named ficolin-1 (M-ficolin), ficolin-2 (L-ficolin) and ficolin-3 (H-ficolin), which are structurally comparable to MBL and C1q.

The final stage of the three complement pathways consists of the formation of the membrane attack complex (MAC). The C5 convertases (C4b2a3b and C3(H_2O)BbP3b) split C5 into C5a and C5b. C5a can bin the C5a receptor (C5aR or CD88). C5b remains bound to the bacterial surface and associates with circulating C6, followed by the formation of a hydrophilic complex. The subsequent binding of C7 to the complex induces a comformational change which exposes the lipophilic groups. This is followed by association of C8 and insertion in the lipid membrane of the target cell. A transmembrane pore can be formed by binding of 10-15 C9 proteins. Insertion of MAC in the target cell membrane can induce osmotic imbalance and results in lysis of the target cell.

Procoagulative activities of complement factors

Several studies have shown that there are links between the complement system and coagulation system. Induction of the complement system after bacterial infections results in enhanced activation of the intrinsic and extrinsic coagulation cascade and decreased fibrinolytic activity (Figure 3). The subsequent formation of a fibrin network keeps bacteria localized to the site of infection.

Enhanced TF expression

It has been shown that complement factors enhance the expression of TF in human endothelial cells, monocytes and neutrophils[37,38]. Ritis *et al.* showed that antiphospholipid antibody-stimulated C5a is capable of inducing TF expression in neutrophils *in vitro* via the C5aR and thereby triggers the extrinsic coagulation pathway[38]. Antiphospholipid antibodies are present in antiphospholipid syndrome (APS) and can activate the complement cascade via the classical pathway. APS is an autoimmune disease accompanied by a hypercoagulative state. Furthermore, it has been shown that the inactive form of the terminal C complex (iTCC; C5b-9), which is incapable of inserting in cellular membranes, can bind endothelial cells and induces FX activation. Activation of FX occurs most likely via TF up-regulation. This shows that inactive products of the complement system are functional in other processes and can induce coagulation[39].

Increased fibrin formation

In addition to the crucial role of MASPs in activation and regulation of the complement system, it has been shown that MASP-1 has thrombin-like activity and may contribute to localized fibrin formation. *In vitro*, MASP-1 can directly cleave fibrinogen and activates FXIII[40]. Krarup *et al.* showed that recombinant MASP-1 uses the same cleavage sites as thrombin in the fibrinogen β-chain and the FXIII A-chain. Although cleavage of both α- and β-chains is necessary for fibrin formation, cleavage of the fibrinogen α-chain by MASP-1 seems to be less specific. Furthermore, the cleavage rate of thrombin is higher compared to MASP-1[40].

In vitro, MASP-2 can promote fibrin clot formation by generation of active thrombin from prothrombin at the membrane surface where MBL or the ficolins are located[41]. It has been shown that fibrin is covalently bound to bacterial surfaces covered with MBL/MASP-2 complexes[41]. However, the activation potential of MASP-2 is low. Krarup *et al.* suggested that limited thrombin activation *in vivo* will induce local fibrin formation, due to the presence of circulating inhibitors. However, this increases the chance that the generated fibrin will be

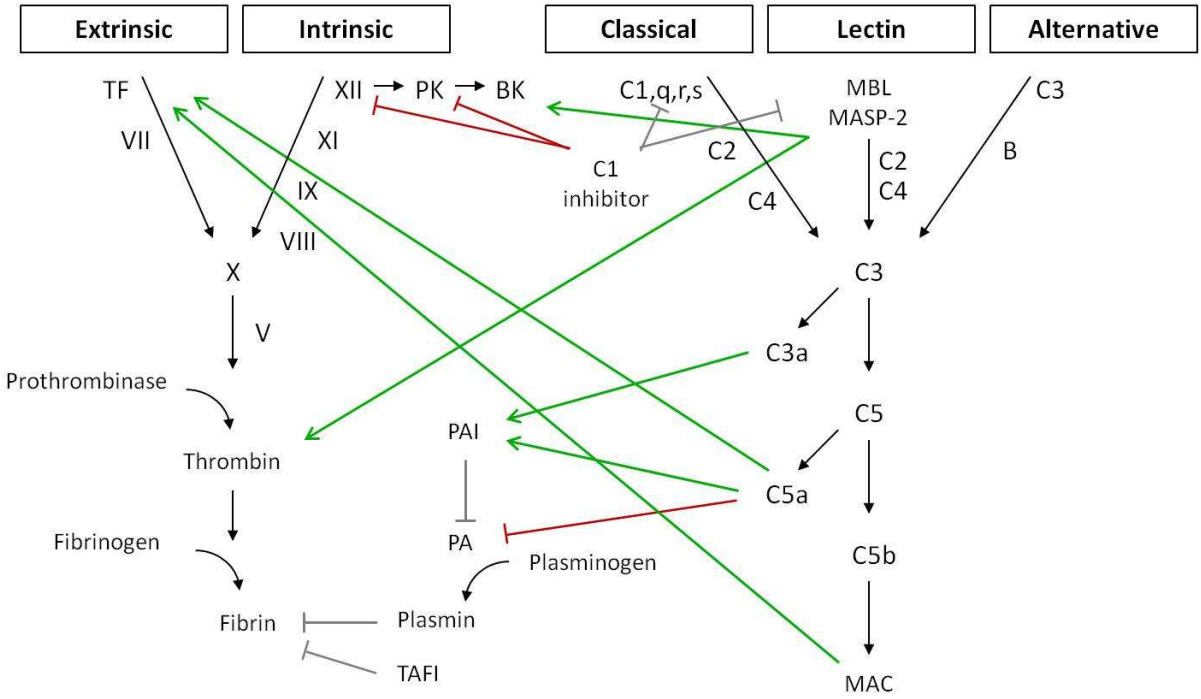

Figure 3. Simplified scheme of the regulation of the coagulation system by components of the complement system. The complement system can both stimulate and inhibit the activity of specific coagulation factors. Activating interactions are represented by green arrows. The red arrows indicate suppressive activity (adapted from Amara *et al.*, 2010[42]).

deposited on the surface to which MASP-2 is bound[41]. Furthermore, activation of fibrinogen is accompanied by the release of the fibrinopeptides A and B, which will attract phagocytes resulting in a proinflammatory state. The role of MASP-3 in inhibition of these processes is unknown. In addition, activation of prothrombin into thrombin by platelets is also catalyzed by the terminal complement complex (TCC; C5b-9)[43].

Inhibition of plamin activation

Recombinant human C5a shows anti-fibrinolytic properties by stimulating PAI-1 production in human mast cells, basophils and monocyte-derived macrophages[44]. The effect of C5a on PAI-1 synthesis is dose- and time-dependent and is directly mediated via C5aR and activation of NFκB[45]. Stimulation with C5a also resulted in a concentration-dependent decrease in t-PA activity[44]. However, the role of C5a in suppression of fibrinolysis *in vivo* remains unclear. In addition, stimulation with C3a induces a slight increase in PAI-1 production in these cells, whereas other complement factors, including C4, C6, C7, C8 and C9 are incapable of stimulating PAI-1 expression[44].

Release of kinins

Complement activation also contributes to the generation of an inflammatory response and release of vasoactive peptides, such as BK, through activation of the kallikrein-kinin system. BK is released after cleavage of HK. HK is often cleaved by PK, but MASP-1 also induces BK release and thereby contributes to the pro-inflammatory effect of the lectin pathway of complement. Both MASP-1 and MASP-2 can cleave HK, but production of BK is only shown for cleavage by MASP-1[46]. Structure analysis showed that the MASP-1 binding groove for HK is similar to that of kallikrein. Furthermore, cleavage of HK by MASPs could be prevented by C1-inhibitor.

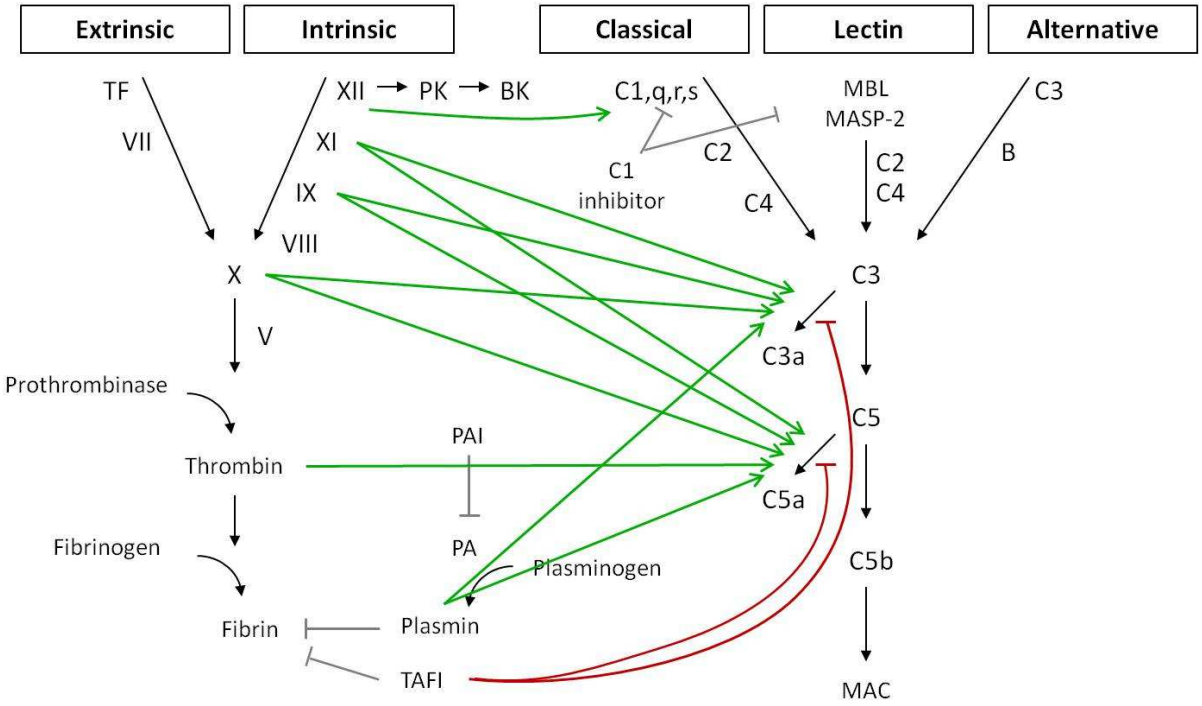

Figure 4. Schematic representation of the activating and suppressive interactions between the coagulation system and complement system. Stimulating activity is represented by green arrows. The red arrows indicate suppressive activity (adapted from Amara *et al.*, 2010[42]).

Complement inhibitors

The complement pathways also share inhibitors with the contact system. In addition to inhibition of activated C1s, C1r and MASP-2, C1-inhibitor suppresses the intrinsic coagulation cascade by inhibition of FXII autoactivaton[47]. This blocks the ability of FXIIa to activate FXI and initiate the intrinsic coagulation pathway. Furthermore, C1-inhibitor also prevents the activation of prekallikrein by FXIIf and subsequent activation of the kallikrein-kinin system[47]. This inhibiting capacity of the complement system thereby contributes to the hemostatic balance.

Complement activation by coagulation factors

There are also coagulation factors that activate the complement system en enhance the inflammatory response, such as thrombin (Figure 4). Thrombin can cleave C3 into C3a *in vitro* in a time and concentration-dependent mechanism[42]. Furthermore, thrombin can generate C5a in the absence of C3[48]. The generation of these anaphylatoxins stimulates the inflammatory response by inducing the migration of phagocytes, degranulation of mast cells and basophils, and the release of vasoactive substances. Similar to thrombin, other proteases have been described to directly cleave C3 and C5, including plasmin, FXa, FIXa and FXIa[42]. However, IXa and FXIa show lower cleavage activity compared to FXA and plasmin. In contrast, TF, FVIIA, FVII and APC failed to activate C3 and C5[42]. Nevertheless, the concentrations used in this study were supraphysiological. Although generation of C3a and C5a by FXa has also been shown *ex vivo* with human serum and plasma, the relevance of these activation pathways *in vivo* is unknown. In addition, it has been shown that the classical pathway can be directly initiated by activation of C1r by FXIIa[47,49]. In contrast, TAFI can remove the carboxy-terminal arginine residue from C3a and C5a, thereby inactivating these components[50].

Conclusion

The coagulation system and its inhibitors maintain the hemostatic balance during injury. Furthermore, factors of the coagulation cascade can modulate the inflammatory response through interaction with PARs and activation of the kallikrein-kinin system. Recently, it has been shown that cleavage of the C-terminus of various coagulation factors releases small peptides with antimicrobial activity against both Gram positive and Gram negative bacteria. C-terminal peptides derived from thrombin, FIX, FX and plasminogen bind to the bacterial surface and induce lysis of the target cell. In addition, these peptides show an anti-inflammatory effects by stimulating the production of IL-10. Although most studies are performed *in vitro* or with mouse models, it has been shown that these host defense peptides are present in skin wounds in humans.

Many pathogenic bacteria have developed mechanisms to recruit and exploit factors of the coagulation and fibrinolytic system for the invasion of host tissue or to escape the physical boundaries of a fibrin clot. Whereas *Staphylococcal* and *Streptococcal* species directly bind and convert plasminogen to plasminogen, *Borrelia* and *Salmonella* express plasminogen-binding receptors and rely on host uPA and tPA to activate plasminogen. The surface-associated plasminogen is used to destruct fibrin networks and extracellular matrix components, which keep the bacteria localized. Furthermore, the binding of plasminogen might decrease opsonization by C3b as was shown for *B. anthracis* and *S. aureus*. Certain bacteria, including *B. fragilis*, express multiple receptors that interact with different stages of the coagulation cascade. In contrast, the protease PgtE from *S. enterica* has multiple binding sites and can interact with either plasmin, α_2-antiplasmin or PAI-1 to modulate the fibrinolytic cascade. In addition, the majority of invading pathogens stimulates the release of the vasoactive component BK by direct or indirect cleavage of host kininogens and thereby induces vascular permeabilization. The recruitment of these coagulation factors, especially kinin release, has stimulated research into new treatment strategies for infectious diseases that target bacterial interactions with these factors.

The activation of the coagulation pathway is accompanied by the activation of the complement system during inflammation. The complement system is an essential innate defense mechanism and simultaneous activation of the coagulation system might effectively inhibit invasion of pathogens. It has been shown that complement factors C3a and C5a induce TF expression on endothelial cells and thereby activate the extrinsic coagulation pathway. Moreover, MASPs do not only initiate the lectin pathway, but generate fibrin by cleavage of thrombin and promote the release of the vasoactive peptide BK from HK. Furthermore, coagulation factors are also able to directly activate C3, which induces the terminal cascade of the complement pathway. These redundant mechanisms might amplificate the activation of both systems and initiate an effective immune response against invading pathogens. However, the extensive interplay between the coagulation and complement systems has consequences for therapeutical interventions in the coagulation system, since interference with the pathway might result in adverse effects in other cascades.

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