The dual role of the contact system in bacterial infectious disease

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Summary
Hemostasis is a sensitive and tightly regulated process, involving the vascular endothelium and blood cells as well as factors of the coagulation and fibrinolytic cascades. Over the last four decades evidence has accumulated that during infection, inflammatory mediators from the microbe and/or host are capable to modulate the equilibrium between the procoagulant and anticoagulant status of the host. Dependent on the mode of activation, these changes can cause either local or systemic inflammatory reactions that may be beneficial or deleterious to the human host. The present review aims to present the state of the art with respect to the role of the contact system (also known as the intrinsic pathway of coagulation or the kallikrein/kinin system) in innate immunity and systemic inflammatory reactions.

Keywords
Coagulation, contact system, inflammation, infectious diseases, innate immunity

The contact system
The contact system comprises four proteins, three proteinases, factor XII (FXII), factor XI (FXI), plasma kallikrein (PK), and a non-enzymatic co-factor, high-molecular-weight kininogen (HK) (Fig. 1). Low-molecular-weight kininogen (LK), an alternative splice product of the kininogen gene, does not belong to the contact system. Under physiological conditions, the four contact factors are either circulating as zymogens in the blood stream or are assembled on various cell types, such as endothelial cells, platelets, and polymorphonuclear neutrophils. Activation occurs when the endothelium changes from an anti-coagulant to a pro-coagulant stage, which is seen for instance upon blood vessel injury. Despite the fact that the system was described by Ratnoff already in 1950’s (for a review see [1]) and has been extensively studied since (for a review see [2]), the molecular mechanisms that evoke an induction of this system on cellular surfaces is still not completely understood. This is in contrast to the activation of the contact system on non-physiological materials, such as kaolin, glass, ellagic acid, and silica (Fig. 2). The initial step here is an auto-catalytically driven activation of F XII, followed by conversion of PK and F XI to their active proteolytic forms by FXIIa. FXIa then triggers the endogenous clotting cascade, whereas activated plasma kallikrein cleaves HK and releases bradykinin (BK). Kinins are important pro-inflammatory mediators that, once they are generated, can lead to the generation of nitric oxide (NO) and other inflammatory mediators, reduction of the blood pressure, induction of fever, and increase of vascular permeability (3).

The observation that patients with deficiencies in FXII, PK, and HK do not suffer from bleeding disorders (4), has led to the current model that contact activation plays only a secondary role in hemostasis. This also holds true for FXII-deficient mice which do not experience spontaneous or excessive injury-related bleeding (5). Interestingly, the animals were found to have a profound defect in the formation and stabilization of platelet-rich thrombi and suppressed bradykinin levels, suggesting an important role for FXII in thrombus formation and inflammation (5, 6). On the other hand, FXI deficiency, also referred to as hemophilia C, can be associated with mild bleeding tendencies, which may for instance occur after surgery (7). The FXI-related bleeding dysfunction can be explained by the ability of thrombin to activate FXI, which is considered an important feedback mechanism to maintain tissue factor-driven activation of thrombin and does not involve the other three contact factors (FXII, PK, and HK).
Based on the observations that the contact system only plays a minor role in hemostasis, it is presently believed that it is mainly involved in the induction of inflammatory reactions, regulation of blood pressure, and sodium homeostasis via the release of kinins (8). As mentioned above, kinins, in particular BK and its metabolite desArg^9BK, are potent inflammatory mediators, causing hypotension, increased vascular permeability, edema formation, fever, and pain (for a review see [9]). The effects of kinins are evoked by their signaling via receptors belonging to the family of G protein-coupled receptors that are predominantly coupled to the pertussis toxin-insensitive Gq type leading to phospholipase C activation, mobilization of intracellular calcium by inositol-1,4,5-trisphosphate (IP3) and activation of protein kinase C (10). In humans, two kinin receptors have been described, designated kinin receptor 1 (B1R) and kinin receptor 2 (B2R) (for a review see [10]). The two receptors have a different expression pattern and pharmacological profile. While B2R is constitutively expressed on most cell types, B1R is normally found in low numbers, but is inducible upon stimulation with pro-inflammatory agents such as interleukin 1β or endotoxin (11). These findings led to the conclusion that the B2R mediates acute inflammatory reactions, while the B1R is involved in triggering a chronic inflammatory response. The two receptors also have a different binding profile. Thus, the B2R interacts preferentially with BK and lys-BK (Lys-BK) is generated by these proteases, For instance upon treatment of LK with tissue kallikrein). It has been reported that B2R activation can also occur by proteases, such as tissue kallikrein, trypsin, or cathepsin G, in a manner as seen for other protease-activated receptors (12). However, these findings have been questioned (10) and it has been suspected that signaling may occur via contaminated kininogens that are processed by these proteases (13). The B1R on the other hand, has higher affinity for BK and lys-BK, which lack the carboxyterminal arginine (10). Under physiological conditions kinin levels are low in human plasma, typically in the lower picomolar range (14). However, in pathological conditions such as vasculitis, children and inflamed joints, kinin levels can be significantly elevated (15, 16).

The contact system and innate immunity

The innate immune system has evolved to recognize conserved structural features of prokaryotic organisms (so-called pathogen-associated molecular patterns, PAMPs). Essential elements here are for instance complement components and antimicrobial peptides (AMPs). At biological boundaries prone to infection, ubiquitously expressed AMPs provide a rapid defense against potentially invasive microorganisms (17–20). Over the last years evidence has accumulated that coagulation and innate immunity have co-evolved, and it is now believed that these systems function as a highly integrated defense mechanism against infectious diseases (for a review see [21]). Thus, the recognition pattern of contact factors functions in a manner that is remarkably similar to that seen in other innate immune systems, for instance the complement system (21). Furthermore, another link between innate immunity and the contact system was recently identified (22). In human plasma, activation of the contact system on the surface of bacterial pathogens, such as S. pyogenes, Staphylococcus aureus and Salmonella, results in processing of HK and generation of highly potent AMPs derived from domain D3 (Fig. 3). The importance of contact activation in bacterial elimination was demonstrated in a mouse model of infection where blocking of contact activation leads to enhanced dissemination of bacteria to the spleens of infected animals (22). A common theme of AMPs is their affinity for heparin (23), and therefore the heparin-binding domain D5 of HK has been studied in respect to its antibacterial activity (24). It was shown that a histidine- and lysine-rich part of D5 exerts potent antibacterial activity against both Gram-positive and Gram-negative bacteria (24). However, in contrast to D3-derived AMPs no generation of AMPs from D5 was observed following contact activation at bacterial surfaces (22), but it was found that neutrophil pro-
teases, as well as elastase from *Pseudomonas aeruginosa*, have the ability to generate antimicrobial peptides from D5 (24) (Fig. 3). The release of BK is also considered as part of the innate immune response. Although the peptide has been reported to be antimicrobial in *vitro* at high concentration (25), a more important function in innate immunity is probably the interaction with alveolar macrophages leading to the release of substances that are chemotactic for neutrophils (26). In addition, kinins have been reported to directly induce migration of neutrophils, involving both B1R and B2R (27). However, neutrophil migration in inflamed tissues was found to be impaired in B1R-deficient mice (28), which was not reported for mice that lack B2R (for a review see [10]). Taken together, these recent studies suggest that the contact system plays an important role in innate immunity.

**Bacteria-induced contact activation**

Apart from plasma and tissue kallikrein, many bacteria-derived proteinases have been described to degrade kininogens and release kinins (Table 1). Microbial proteinases can also release BK indirectly through activation of FXII and/or PK, which subsequently cleave kininogens and release kinins (Table 2). This has been suggested as a mechanism to increase the vascular permeability promoting an influx of plasma containing nutrients into the site of infection and help spreading the infection via the microcirculation. In addition, components of the contact system have been shown to assemble at the surface of several important pathogens, leading to an activation of the system (Fig. 3), whereby BK is also released (29). The ability of structural elements, such as the negatively charged lipopolysaccharides (LPS) of Gram-negative bacteria and teichoic and lipoteichoic acid of Gram-positive bacteria, to activate the system was reported by Kalter et al. as early as 1985 (30). Bacterial surface proteins and virulence determinants, like the M proteins of *Streptococcus pyogenes* and curli fibers expressed by *Escherichia coli* and *Salmonella*, were later found to bind and assemble the contact factors resulting in BK release (31–34). A massive activation of the contact system in the circulation may lead to pathological levels of kinins and a consumption of contact factors, contributing to the hypovolemic hypotension and coagulopathy of sepsis and septic shock. This is underlined by many clinical studies conducted over the last forty years (for a review see [35]), and already in 1970 Mason et al. published that patients with hypotensive septicemia have significantly decreased levels of contact factors (36). It is now clear that low levels of FXII and HK in patients with SIRS (systemic inflammatory response syndrome) correlate with a fatal outcome of the disease (37). Pronounced contact activation has also been reported in children with meningococcal septic shock (38, 39) and in patients with streptococcal toxic shock syndrome (40). Interestingly, patients suffering from streptococcal toxic shock syndrome who have prolonged clotting times of contact system-driven and normal tissue factor-driven coagulation, show no bleeding disorders (40). Similar findings were also made in animal experiments where contact activation was studied in a *E. coli* sepsis model in baboons (41). In this case blocking of FXII activity did not protect from complications due to blood coagulation, but hypotension was prevented. The conclusion was that the generation of kinins plays an important role in hemodynamic changes during sepsis (41). Kinin concentration in septic patients can reach levels that exceed the lower nanomolar range.

| Table 1: Bacterial proteinases that trigger the release of kinins from kininogens. |
|------------------------|------------------------|------------------------|
| **Bacterial species**   | **Enzyme**             | **Reference**          |
| *Porphyromonas gingivalis* | Lys-gingivain         | (62)                   |
| *Staphylococcus aureus*  | Stechapain            | (63)                   |
| *Porphyromonas gingivalis* | PK/HK                 | (66, 67)               |
| *Pseudomonas aeruginosa* | FXII (65)             |                        |
| *Serratia marcescens*   | FXII (65)             |                        |
| *Streptomyces proteinase* | FXII/PK              | (65)                   |
| *Streptomyces coelicopitans* | FXII/PK              | (65)                   |

| Table 2: Bacterial proteinases that activate contact factors. |
|------------------------|------------------------|------------------------|
| **Bacterial species**   | **Target**             | **Reference**          |
| *Aspergillus fumigatus*  | FXII (65)             |                        |
| *Bacillus subtilis*     | FXII (65)             |                        |
| *Pseudomonas aeruginosa* | FXII (65)             |                        |
| *Serratia marcescens*   | FXII (65)             |                        |
| *Vibrio cholerae*       | Not known             | (69)                   |
| *Vibrio parahaemoliticus* | FXII/PK              | (70)                   |
| *Vibrio vulnificus*     | FXII/PK               | (65, 70)               |

**Figure 3: Contact activation on bacterial surfaces.** Following assembly of the contact factors on bacterial surfaces, HK is processed and releases antimicrobial peptides (AMPs) from domain D3. AMPs from HK domain D5 can also be released through the action of neutrophil proteinases. As a consequence released AMPs kill the bacteria.
(42), which is sufficient to evoke an activation of the two kinin receptors (10). Apparently, B1R and B2R are regulated differently in response to infection. For instance, it was found that stimulation with *Burkholderia cenocepacia* triggers an up-regulation of B1R, but not B2R, in human fibroblasts (43). Similar discoveries were made (see also Fig. 4) when tissue samples from patients suffering from soft tissue infections caused by *S. aureus*, were examined by immuno-histochemistry (44). Other studies have shown that B1R and B2R are upregulated in murine tracheal muscle cells upon stimulation of TLR4 (toll-like receptor 4) and TLR3 (45).

**Inhibition of contact activation: Clinical perspective**

Pharmacological agents that interfere with the effects of contact activation on microcirculation and blood pressure during inflammation and sepsis are of obvious clinical interest, and a number of kinin receptor antagonists have been developed and used in various animal models (for references see [46, 47]). However, only few human clinical studies have been reported so far. For example, the efficacy of the potent B2R antagonist HOE 140, also known as Icatibant (47), was studied in patients with asthma and acute rhinitis, where the effects caused by BK were inhibited (48, 49). Although, the effect of HOE 140 has not been shown in septic patients, animal studies demonstrate that this antagonist attenuates vascular permeability caused by LPS (50). Non-peptide B2R antagonists represent another group of compounds potentially suitable as therapeutic agents in allergic airway disease (51). Yet, there are no human clinical studies on these compounds, but their efficacy has been demonstrated in several animal models of airway inflammation (51). Deltibant, that acts on B2R, is so far the only kinin antagonist which has been used in clinical trials for sepsis (52). In a multicenter, randomized, placebo-controlled trial, the drug was applied to patients with SIRS and presumed sepsis. Even though the drug had no significant effect on risk-adjusted 28-day survival, post-hoc analysis revealed a non-significant trend toward improvement (52). More recently a generation of non-peptide molecules acting as selective B1R antagonists have been developed and successfully used in experimental animal studies for treatment of inflammatory conditions and pain (53).

The major regulator of contact activation is C1-esterase inhibitor (C1-Inh), which inactivates PK, FXIIa and FXIa (54, 55). C1-Inh is used as a replacement therapy for patients with hereditary angioedema, a condition caused by a deficiency of C1-Inh. A potent and selective inhibitor of PK is ecclantanide (DX-88), which in a clinical study significantly improved the symptoms of patients with hereditary angioedema. A phase III trial is now ongoing (56). The C1-Inh can be inactivated by neutrophil elastase and it has been demonstrated that the concentration of proteolytically inactivated C1-Inh is increased in septic patients as compared to healthy controls (57). Administration of C1-Inh to patients with severe sepsis interfered with contact activation and showed beneficial effects on hypotension and renal dysfunction (58–60). Also, in animal experiments systemic effects and vascular permeability, caused by LPS, were reduced by C1-Inh (61), suggesting a potential role for this regulator of contact activation in treatment of inflammatory diseases.
Concluding remarks

It may appear contradictory that the contact system, a branch of innate immunity, protecting against invasive bacteria, is partly responsible for the hypovolemic hypotension typical of septic shock. However, bacterial pathogens are masters of manipulating various host defenses (complement, immune, and coagulation systems), and it is well established that exaggerated responses by these defense systems to pathogens, create a proinflammatory state which is mainly responsible for many of the symptoms seen in patients with severe infectious disease. In a situation where a systemic and massive activation of the contact system contributes to the sepsis syndrome, an early and efficient inhibition of the contact activation should be an attractive therapeutic strategy, although the generation of antibacterial peptides by the contact system is also blocked.

References


