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The Protease Storm of Angioedema

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ABSTRACT

Attacks of angioedema are hallmarked by edematous swelling of skin and mucosal tissues, which are painful and sometimes life threatening. The molecular mechanisms involved in the initiation and propagation of these attacks are poorly understood. During attacks, a storm of proteolytic activity is generated that mediates this disease. Deficiency in C1 esterase inhibitor is a strong risk factor, but there are many patients who experience angioedema without a known cause. This suggests that one or more elusive factors beyond this deficiency increase the susceptibility for developing this disease. Several lines of evidence suggest that the plasma contact system plays an active role in angioedema. This enzyme system is well known for its capacity to trigger blood coagulation *in vitro*, but its physiological role *in vivo* is unknown. The plasma contact system is also functionally linked to the complement system and fibrinolytic system, both of which are implicated in angioedema. Furthermore, the contact system is responsible for the liberation of kinins from the plasma protein high-molecular-weight kininogen. Recent findings from fundamental studies have identified endogenous activators of the contact system that liberate kinins without concurrent activation of blood coagulation. Interestingly, clinical evidence suggests a similarity with patients with angioedema: pathologic amounts of kinins form without concurrent clinically overt thrombotic pathology. Detailed biochemical studies are ongoing to explain the apparent preference of the plasma contact system for kinin formation in angioedema. This improved understanding of the functioning of the contact system may help pinpoint therapeutic targets. Finally, recent technologic advances have led to the development of analytical methods for determining the activity of contact system enzymes. It is hoped that the development of new bioassays will improve diagnosis and guided personalized treatment of patients with angioedema.

INTRODUCTION

The term “cytokine storm” is commonly used to describe the excessive release of cytokines and their complex activities in immune-related conditions, such as graft-versus-host disease, pancreatitis, and infectious diseases.[1] Popularly, it is also used to describe the unpleasant feeling that precedes the flu. In comparable fashion, itching or painful sensations, outbreaks of hives or other signs, such as lowering of the voice, can reportedly precede attacks of angioedema. These signs may warn experienced patients about oncoming attacks. Meanwhile, in the blood of patients, uncontrolled enzymatic activity and the generation of kinins hallmark attacks of angioedema. Most notably, activation of the complement system, fibrinolytic system, and plasma contact system take place. While the complement system is responsible for host defense, fibrinolysis is needed to degrade and tone blood clots after injury. The physiological role for the plasma contact system is unknown, but it has been implicated in thrombotic disease,[2-5] allergic vascular leakage,[6] and hypotension during experimental studies on septicemia.[7] As is the case for the cytokine storm in infectious disease, it is complicated to establish a general causative factor in the onset of angioedema attacks. At first glance, either excessive enzymatic activity or an increased sensitivity toward kinins or complement factors may be the difference between health and disease. However, either one of these possibilities does not necessarily rule out the other.

PLASMA CONTACT SYSTEM AND COAGULATION

When blood contacts foreign materials, such as glass, certain plastics, negatively charged polymeric molecules, and precipitated antioxidants (i.e. ellagic acid[8]), plasma coagulation follows.[9] To prevent contact-triggered coagulation, diagnostic blood sampling tubes contain Ca^{2+}-chelating anticoagulants or heparin. Furthermore, it is of clinical interest that implantable biomaterials,[10] nanoparticles,[11,12] and therapeutic agents[13] are compatible with the contact system to prevent adverse reactions in patients. Contact-triggered coagulation requires the activation of the intrinsic pathway of coagulation. This initiates when factor XII (FXII) spontaneously activates (into FXIIa) after binding to the incompatible materials. In a second step, factor XI (FXI) is activated by FXIIa on the surface and coagulation is triggered. Basic properties of the contact factors are summarized in the **table**. For proper activation, FXI needs to attach to incompatible materials via its natural cofactor high-molecular-weight kininogen (HK). As a result, contact-triggered plasma coagulation is impaired when FXII, FXI, or HK is lacking. Simultaneous to the activation of FXI, FXIIa activates plasma prekallikrein (PPK) into kallikrein (PK). While there are 15 tissue kallikreins, PPK has no family members in plasma. However, PPK is strikingly homologous to FXI[14] and also circulates and binds to surfaces in complex with HK.[15,16] Although all FXI circulates with HK, ~25% of PPK remains free in the circulation. PK activity has 2 roles in contact-triggered coagulation: 1) it cleaves surface-bound

FXII and PPK, and 2) it cleaves surface-bound HK. These 2 types of cleavages stimulate the activation rate of FXII and the procoagulant activity of HK, respectively. As a result, PK deficiency slows contact-triggered coagulation *in vitro*, which can be corrected by prolonged exposure of blood to incompatible materials.[17] This indicates that PPK is a catalyst for contact-triggered coagulation.

Coagulation induced by contact activation was originally identified as a process that solely occurs *in vitro* and is useful to investigate clotting defects such as hemophilia, screen for heparin, or detect lupus anticoagulant activity. This is supported by the observation that deficiencies in contact factors do not lead to bleeding phenotypes.[18] Furthermore, lessons can be learned from deficiency in C1 esterase inhibitor (C1inh). This serine protease inhibitor is important for the inactivation of complement factors C1r and C1s, as well as FXIIa, FXIa, and PK. Although the contact system proteins are essential to coagulation *in vitro*, C1inh deficiency is not associated with a prothrombotic state (unlike, for example, antithrombin III deficiency). However, an increasing body of evidence from animal studies indicates that FXII-dependent coagulation does play a role in thrombosis,[2,3] by excessive reinforcement of occluding thrombi.[19] At present, the contact factors are considered promising targets for the safe management of thrombosis. Indeed, inhibition of contact system inhibitors and targeting their expression reversed a thrombotic phenotype in various experimental animal models.[4,5,20,21] However, the possibility still exists that contact system-related coagulation is needed to fine-tune clot stability under arterial flow rates,[22] and that targeting the contact system may increase the risk of developing embolic events. Hence, it will be important to investigate whether contact system inhibitors can safely be used to manage thrombotic disorders in humans.

PLASMA CONTACT SYSTEM AND KININS

When PK cleaves HK, the small (9-amino acid) peptide bradykinin is released. Bradykinin has the sequence Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, which strongly resembles the 10-amino acid peptide kallidin with the sequence Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (aka, Lys-bradykinin) that is released from low-molecular-weight kininogen by tissue kallikreins. At present, it is generally assumed that bradykinin mainly is a product of intravascular kallikrein activity, whereas kallidin is a product of tissue kallikrein activity. This is supported by the observation that bradykinin levels are reduced (but not absent) in mice that are FXII deficient.[23] However, both bradykinin and FXIIa have been identified in the cerebrospinal fluid of patients with Alzheimer's disease, indicating that contact system activation can occur outside the vasculature.[24,25] Kallidin and bradykinin can both activate the kinin B2 receptor (B2R) on endothelial cells. While B2R is constitutively expressed, B1R expression is upregulated after leukocyte activation during tissue injury and mediates inflammatory responses.[26] Activation of B2R leads to vasorelaxation, which occurs through the G-protein–mediated production of nitric oxide by endothelial cells that instructs underlying smooth muscle cells to relax. As a result, fluctuation of bradykinin levels can directly affect blood pressure.

Several peptidases are able to cleave bradykinin and kallidin, including angiotensin-converting enzyme (ACE), carboxypeptidase N, and aminopeptidase P.[27] Carboxypeptidase N removes the C-terminal arginine from bradykinin and kallidin. This reduces the affinity of these peptides for B2R but enhances their affinity for B1R.[28] ACE is considered important for the inactivation of kinins, as it is abundantly present in lung endothelial cells. In a first step, it can cleave the Pro-Phe bond at position 7-8 of bradykinin (8-9 in kallidin). Subsequently, it can further

Table. Basic Parameters of the Contact System Components

Protein	Weight	Concentration	Concentration
Factor XII	78 kDa	30 µg/mL	375 nM
Prekallikrein	86 kDa	50 µg/mL	580 nM
High-molecular-weight kininogen	110 kDa	50 µg/mL	636 nM
Factor XI	160 kDa	5 µg/mL	31 nM
C1 esterase inhibitor	105 kDa	250 µg/mL	3.33 µM

cleave the remaining peptide at Phe-Ser 5-6 (6-7 in the kallidin-related degradation product). While active kinins have a very short half-life (estimated 10-50 seconds), their degradation products are more stable and can be detected up to ~1 hour after administration.[29,30] Interestingly, therapeutic inhibition of ACE for the management of hypertension elevates plasma bradykinin levels, which may be partly responsible for the immediate blood pressure–lowering effects of these drugs. However, ACE inhibition also has negative effects: development of a characteristic dry cough is a described adverse effect of ACE inhibitor use, which is thought to be mediated by bradykinin.[31] A more rare adverse effect of ACE inhibitor use is angioedema, which is clinically indistinguishable from attacks of hereditary angioedema. What factors predispose specific patients on ACE inhibition for development of these 2 kinin-mediated adverse effects is unknown, but cessation of therapy effectively prevents further problems.

CONTACT SYSTEM ACTIVATION IN ANGIOEDEMA

During angioedema attacks, uncontrolled activation of FXII and PPK result in the cleavage of HK and formation of bradykinin[32] (and its metabolites). Loss of control over contact system activity may occur as a result of C1inh deficiency or by gain-of-function mutations in FXII and suggests that the contact system is involved in the onset of angioedema attacks.[33] Furthermore, plasminogen activation and complement activation have been reported, which are often considered to be secondary to contact activation. It is of interest that antifibrinolytic agents such as tranexamic acid have long been known to ameliorate symptoms in patients with nonhereditary angioedema, indicating an active contribution of plasmin to disease.[34] Because FXIIa can activate plasminogen,[35] and plasmin can generate FXIIa from its precursor,[36] it is possible that therapeutic inhibition of this enzymatic positive feedback loop effectively restores the control. Elevated thrombin-antithrombin (TAT) and F1+2 complexes have been reported in hereditary angioedema.[37,38] surprisingly, these are possibly not related to FXII-driven coagulation, as no evidence for FXI activation was found in these patients.[37] It is possible that the observed TAT complexes in angioedema are triggered by tissue factor, which becomes exposed to plasma during its extravasation or as a result of neutrophil activation. Alternatively, it is possible that the procoagulant activity of FVII rises during attacks of angioedema, through cleavage by FXIIa.[39] It is intriguing that contact activation *in vivo* appears dissimilar from contact system activation *in vitro*, which is characterized by the generation of a strong procoagulant response (despite the presence of plasminogen in plasma). Indeed, direct evidence of an association between angioedema and thrombosis is scarce, and often accompanied by significant independent prothrombotic risk factors.[40-42] Taken together, this suggests that the mode of contact system activation during attacks of angioedema differs from the mechanism of contact activation *in vitro,* which leads to plasma coagulation.

NEWLY IDENTIFIED TRIGGERS FOR CONTACT SYSTEM ACTIVATION

Despite the increasing amount of evidence that angioedema is a protease-mediated disease, the trigger factors for activation of these systems remain to be elucidated. From clinical observations, diverse triggers have been reported, including mild tissue injury[43] and allergy.

Misfolded protein aggregates

In an interesting patient study, experimental injuries were applied to the skin of patients with hereditary angioedema.[44] Subsequently, the fluid in the provoked blisters was analyzed. Although the injuries themselves did not directly evoke signs of urticaria or angioedema in these patients, the blister fluid was shown to contain active kallikrein. This indicates that the contact system can become directly activated in response to tissue injury, also when induced outside the vasculature. In injured tissues, a number of candidate contact system activators present themselves. These may include microparticles from apoptotic cells[45] and anionic phospholipids.[46] However, in 2002, a novel immunologic concept proposed that damaged and aggregated proteins may provide a general danger signal for the (innate) immune system.[47] Indeed, protein aggregation is a known trigger factor for development of antibodies against protein drugs and autoantigens.[48-50] Of note, protein aggregation-related danger signals are also displayed by pathologic protein species in protein deposition diseases, such as Alzheimer's disease. Earlier *in vitro* studies had reported that amyloid-β peptide activates the contact system.[51] Moreover, contact system activity was detected in the cerebrospinal fluid of patients with Alzheimer's.[24] Subsequently, we hypothesized that FXII has the natural capacity to recognize damage-associated molecular patterns (DAMPs) in misfolded protein aggregates and autoactivate in their presence. We investigated this hypothesis by preparing a large number of proteins in their native and misfolded state (produced by pH changes, heat denaturation, formation of advanced-glycation end products, or spontaneous aggregation of pathogenic amyloidogenic peptides). Next, we found that FXII-dependent PPK activation was triggered by all proteins that were misfolded and aggregated, but not by their native counterparts[52] (additional examples can be found online in supplemental data doi:10.1172/JCI35424DS1). In other studies, we had mapped the binding site in FXII and its homologues for misfolded protein aggregates to the fibronectin type I domain.[53] We then investigated our hypothesis *in vivo*, by determining the levels of FXIIa, as well as FXIa-C1inh and PK-C1inh (which reflect recent activation of FXIa and PPK) in systemic amyloidosis. This

disease is characterized by the presence of circulating misfolded protein aggregates, which are unrelated to amyloid-β peptide. We found a significant increase in FXIIa levels, which corresponded with increased PK-C1inh complexes. Surprisingly, there was no evidence that FXI activation occurred in these patients. In comparative *in vitro* experiments, we found that misfolded protein aggregates were unable to trigger FXII-dependent FXI activation. In further studies, we identified that plasminogen activation also occurred in patients with systemic amyloidosis.[54] Together, these findings indicate that FXII-dependent kallikrein formation may play a role in the early recognition of DAMPs, presented by misfolded protein aggregates. Interestingly, this occurs in a pattern that resembles angioedema attacks (ie, FXII, PPK, and plasminogen activation without concurrent FXI activation). As angioedema is reportedly triggered by tissue damage, misfolded protein aggregates may form a relevant trigger factor.

Mast cells and heparin

Allergy is another reported trigger for angioedema attacks. Based on clinical experience, angioedema can be subdivided into histaminergic and nonhistaminergic angioedema. This suggests that histaminergic angioedema is completely unrelated to contact system activation. Indeed, there has been negative evidence for systemically circulating bradykinin in patients with histaminergic angioedema , while bradykinin was either detected locally or systemically in patients with various other types of angioedema.[55] This leads to the question of how 2 separate mechanisms of disease can present themselves in a similar phenotype. Evidence from recent studies offers a consolidating explanation and points to kinin formation as a common step in the development of angioedema. Mast cells are key mediators of allergic reactions. During their degranulation, they release a number of important allergic mediators, including histamine and tryptase. One characteristic component of mast cell granules is heparin, which is held responsible for proper storage of mast cell contents.[56] In previous studies, it was shown that a multitude of negatively charged polymers can activate the contact system, including platelet polyphosphates,[3] extracellular RNA,[57] and artificial anionic compounds such as dextran sulfate.[58,59] As heparin also is a highly negatively charged glycosaminoglycan, the hypothesis was raised that this compound directly activates the contact system. Using heparin that was directly isolated from activated peritoneal mast cells, this hypothesis was confirmed *in vitro*.[6] Interestingly, although mast cell heparin triggers FXII-dependent bradykinin formation, it does not trigger activation of FXI. In parallel, *in vivo* mouse studies revealed that ~50% of contact system–driven bradykinin formation could be attributed to allergy-related hypotension and vascular leakage. Therapeutic interference with bradykinin-mediated signaling ameliorated mast cell–mediated vascular leakage, whereas it was exacerbated in Serping1$^{-/-}$ (C1inh deficient) mice. These findings suggest that histaminergic and bradykinin-dependent angioedema are not necessarily mutually exclusive in (hereditary) angioedema. Both triggers for contact activation described above provoke kallikrein formation without coagulation.

TWO DIVIDED MODES OF CONTACT ACTIVATION

Activated FXII has 2 activities: First, it can act as a coagulation factor by activating FXI. Second, it can act as an activator of the kallikrein-kinin system via PPK activation. During *in vitro* contact activation, 2 scenarios have been identified

Simultaneous activation of coagulation and bradykinin formation

From *in vitro* experiments, it has become known that materials such as kaolin and ellagic acid, which trigger coagulation, also simultaneously provoke the PK-dependent degradation of HK. **Figure 1** shows that kaolin, at concentrations that can also be used to trigger coagulation,[60] effectively induces degradation of HK. This activation is highly dependent on PPK; with decreasing amounts of plasma PPK, the capacity for HK cleavage diminishes progressively. It is of note that a small amount of HK always appears cleaved in unstimulated plasma (indicated by “vehicle”). This corresponds to a constant turnover of HK and continuous liberation of bradykinin in the circulation.[23] At present, there are only a limited number of known materials that are able to evoke FXII-dependent coagulation (reviewed in Maas et al[9]). These are often non-endogenous and insoluble (e.g. kaolin and ellagic acid[8]). A subselection have been reported to be involved in thrombosis *in vivo*,[3,57] but their role in hemostasis is limited.

Unilateral activation of the kallikrein-kinin system

On the other hand, there are a number of activators of FXII and the contact system that do not activate coagulation at all. This is very surprising, as the contact system is mainly known as part of the coagulation system. In response to triggers such as dextran sulfate, mast cell heparin, and misfolded protein aggregates, FXII-dependent PK formation and HK cleavage proceed independently from coagulation. It appears that the kallikrein-kinin system and FXII-dependent coagulation can functionally compete for each other: the exposure of plasma to kallikrein-specific activators strongly diminishes the capacity for FXII-dependent coagulation. This was recently demonstrated for dextran sulfate.[60] In an extension to this finding, Figure 2 shows that misfolded protein aggregates compete for coagulation in a very similar manner. Misfolded aggregates of bovine serum albumin (but not native protein) dose-dependently increase both kaolin-triggered coagulation times (Fig. 2A) and recalcification times (Fig. 2B). An important question

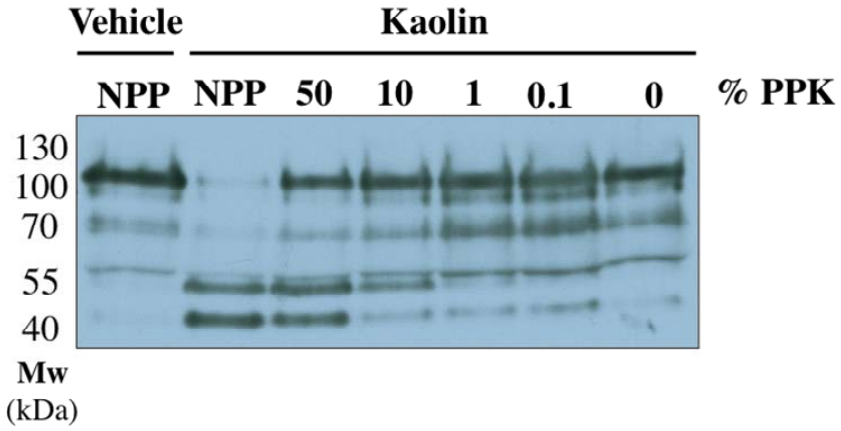

Figure 1. Plasma high-molecular-weight kininogen (HK) cleavage. Normal citrated plasma (NPP) was incubated for 15 minutes with saline only (vehicle) or 150 µg/mL of kaolin. Plasma prekallikrein levels were modulated by mixing NPP with congenital prekallikrein (PPK)-deficient plasma before the experiment. Western blotting was performed according to standard procedures on 12% acrylamide gels, blotted onto polyvinylidene difluoride membranes. HK was detected using a polyclonal antibody.

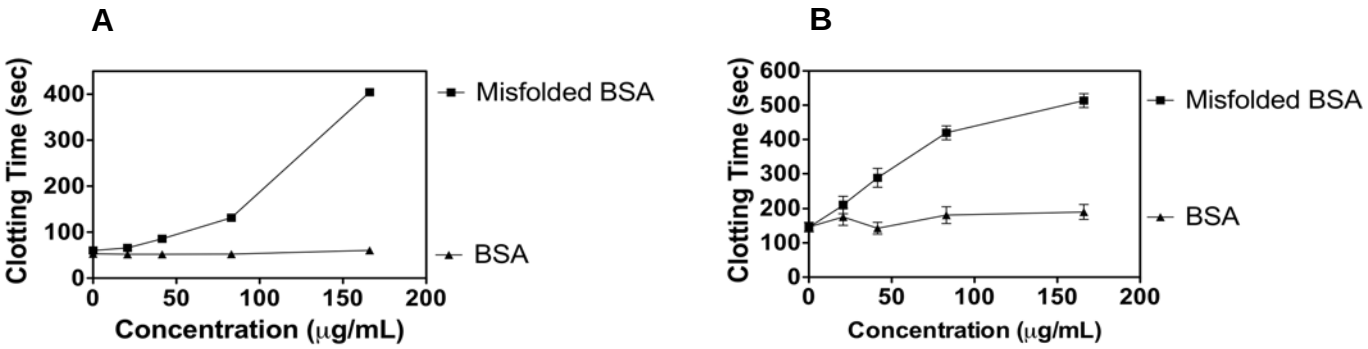

Figure 2. Misfolded protein aggregates interfere with factor XII–dependent coagulation *in vitro*. (A) Kaolin-induced coagulation and (B) recalcification times in the presence of a concentration range of misfolded or native bovine serum albumin (BSA; prepared by glycation as described in Maas et al[52]). In brief, normal pooled plasma was spiked with misfolded or native BSA, 5 minutes before the experiment. Subsequently, (A) 150 µg/mL of kaolin or (B) buffer was added and incubated for 2 minutes. Plasma coagulation was induced by the addition of 10 µM of PS:PC:PE vesicles (prepared as described in Maas et al[72]) and 8.3 mM of $CaCl_2$. Data shown are experiments performed in triplicate with standard deviations.

that results from these findings is: How can the contact system activate the kallikrein-kinin system without simultaneous coagulation? The answer may reside in the biochemical characteristics of FXII.

PROGRESSIVE PROTEOLYSIS OF FXII DURING ACTIVATION

The single-chain 78-kDa FXII zymogen requires a primary cleavage at residue Arg^{353} to become activated[61] (Fig. 3). Without this cleavage, FXII remains inactive, indicating that Arg^{353} is the main "on-off switch" for FXII. After this first cleavage, FXIIa does not fall apart. In contrast, the 2 peptide chains are held together by a disulfide bond and the molecule retains its original molecular weight of 78 kDa. This molecule is titled αFXIIa and is able to bind to activating materials through its heavy chain. Furthermore, it can activate both FXI and PPK. The heavy chain contains a direct binding site for FXI that is involved in its activation.[62] In further cleavages, the FXIIa heavy chain and light chain (comprising the protease domain) become separated. The released 28 kDa protease domain is named βFXIIa, and has lost the capacity to activate FXI or bind to surfaces. βFXIIa is a poor activator of FXI, both when it is surface bound and in solution, but is an effective activator of PPK, irrespective of its location.[63] Considering the strong homology between FXI and PPK, it is surprising that FXI requires binding to the FXIIa heavy chain, whereas PPK does not. Based on the apparent selectivity of αFXIIa and βFXIIa for their substrates, it is presently attractive to hypothesize that contact system activators

that trigger unilateral activation of the kallikrein-kinin system (e.g. mast cell heparin and misfolded protein aggregates) evoke rapid generation of βFXIIa. In contrast, activators that are able to trigger coagulation will evoke a slower progressive proteolysis and retain intermediate forms of FXIIa (ie, αFXIIa) that can activate FXI. During angioedema attacks, it appears that FXIIa has limited capacity to directly induce coagulation.[37] This suggests the generation of specific isoforms of FXIIa that only support PPK activation.

NEW TOOLS FOR QUESTIONING THE CONTACT SYSTEM IN ANGIOEDEMA

The activities of the contact system components have previously been charted with a number of sensitive tools. Assays have been developed for the determination of plasma levels of FXIIa[64] and FXIa,[65] as well as FXIa-[66], FXIIa-, and PK-[67] complexed to C1inh and other inhibitors. Intuitively, the prevention of artifactual post-sampling contact activation is required for accurate determinations with these assays. To achieve this goal, some groups have added polybrene[67] or aprotinin[68] to anticoagulated plasma samples for their studies on contact factors, while (cleaved) kininogen levels were previously determined in a cocktail of protease inhibitors.[69] Studies of contact system activation have also successfully been performed in citrated plasma without additional inhibitors: for instance, FXIIa has been found to predict long-term mortality in patients admitted with chest pain.[70] However, detection limits in this FXIIa assay benefit greatly from the presence of 3:4-dichloroisocoumarin and Triton X-100, indicating that comparable studies may experience great advantages from dedicated sampling.

Currently there is a growing call for insightful diagnostic assays to either identify or exclude a role for deregulated contact system activity in patients with (idiopathic) angioedema. Although FXIIa-C1inh and kallikrein-C1inh complexes are very informative for determining contact system activation in general, both quantitative and qualitative C1inh deficiencies (or gain-of-function mutations in FXII) could complicate such determinations for diagnostic purposes in angioedema. Ideally, the detection of free contact system enzymes at the time of attack, as well as the levels of kinins, would provide the most relevant diagnostic information. Nanobodies are recombinantly produced monovalent camelid-derived antibody fragments of ~10% the size of a conventional monoclonal antibody. They can be rapidly selected and characterized, through use of phage-display technology. Because of their small size, they are able to probe conformational changes in circulating proteins. As a result, they are potentially useful for the generation of diagnostic assays.[71]

We recently hypothesized that different forms of FXIIa are generated in response to various contact system activators. To investigate this hypothesis, we generated nanobodies against the protease domain of FXIIa. Two nanobodies were selected for their ability to recognize FXIIa, but not FXII zymogen.[60] Nanobody A10 recognizes

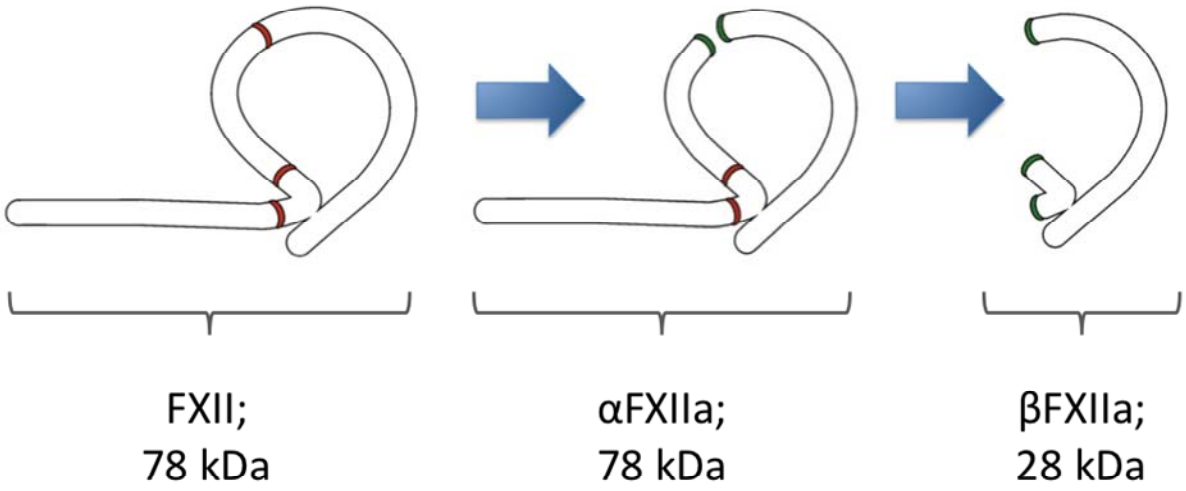

Figure 3. The stepwise proteolysis of factor XII (FXII) during activation. FXII zymogen has several cleavage sites for trypsin-like activity, indicated by the red rings. It is composed of a stretch of surface-binding domains and a loop that harbors the inactive protease domain. After a primary cleavage at Arg^{353}, the enzyme retains full size (held together by a disulfide bond) and can activate both plasma prekallikrein and FXI. Further proteolytic steps liberate the light catalytic domain from the heavy surface-binding chain. This enzyme can still activate prekallikrein, but no longer supports FXI activation.

the catalytic domain of purified αFXIIa, but not that of purified βFXIIa. However, nanobody B7 recognizes both forms, suggesting minute differences in the catalytic domain between these 2 isoforms of FXIIa that can be distinguished by these nanobodies. The capturing of FXIIa from human plasma by nanobodies is strongly enhanced in the presence of the small-molecule serine protease inhibitor PPACK. This molecule occupies the FXIIa active site and prevents the association of macromolecular plasma protease inhibitors, such as C1inh, which disrupt nanobody recognition of FXIIa. During contact system activation in plasma *in vitro*, nanobody-based capture assays distinguish activation products of FXII that vary with the type of activator present: kaolin, a procoagulant contact system activator, solely triggers the formation of a species that is captured by B7. In contrast, dextran sulfate, which triggers unilateral activation of the kallikrein-kinin system, first generates a species that is recognized by B7, which is later converted into a second species that is recognized by A10.

OPPORTUNITIES FOR FUTURE MANAGEMENT OF ANGIOEDEMA

New contact system activators have recently been identified that may be involved in the onset of angioedema attacks and may represent trigger factors such as tissue injury and allergy. In the presence of these activators, the kallikrein-kinin system becomes activated without simultaneous coagulation. At present, the mechanism behind this remarkable mode of kinin-specific contact activation remains to be elucidated. However, it may relate to the capacity of FXIIa to form multiple species. Because tissue injury, allergies, or ACE inhibitor use (fortunately) only trigger angioedema in a subset of the general population, it is expected that a number of susceptibility factors (beyond C1inh deficiency) modulate the risk for developing attacks. It will be very interesting to determine those additional susceptibility factors. Recent findings with a new nanobody-based bioassay for FXIIa suggest that progressive proteolysis converts FXIIa from a coagulation factor into a specific activator of PPK. This may help to identify the exact molecular species of FXIIa that trigger the excessive kinin formation in angioedema attacks. Ultimately, the development of robust bioassays for the quantitative determination of FXIIa, PK, and kinins (or alternatively, cleaved HK) may aid our understanding and therapeutic management of this enigmatic and dangerous disease.

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CONFLICT OF INTEREST

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