

# Contact System Activation in Patients with HAE and Normal C1 Inhibitor Function

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## KEYWORDS

• Factor XII • Kallikrein • Kininogen • Kinin forming enzymes • Contact phase

## KEY POINTS

- Contact phase proenzymes are activated in conditions when endothelium is triggered with diverse materials and under various conditions.
- The proenzyme to enzyme conversion is under the control by serpins, and proteolytic activity develops toward high-molecular-weight kininogen (HK).
- Hereditary angioedema with normal C1 inhibitor function (HAE-nC1INH) is associated with this biologic condition and, in a few cases (<10%), a missense mutation in the *F12* gene.

## INTRODUCTION: NATURE OF THE PROBLEM

Kinin-mediated angioedema (AE) is a rare disorder characterized by recurrent and unpredictable episodes of localized swelling (face, extremities, bowel walls, genitals, and upper airways).<sup>1,2</sup> Occurring at the endothelium/plasma interface, this vascular permeability depends on the kinins (bradykinin [BK] and *desArg*<sup>9</sup>-BK) released on proteolytic enzymes on the precursor HK during activation of kallikrein (KK)-kinin system

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(alternatively described as contact system).<sup>1–4</sup> Plasma KK mainly supports the proteolytic activity toward HK.<sup>5</sup> With the renin-angiotensin system, the KK-kinin system represents the prototype of successful enzymatic mechanism elaborated during evolution for the control of vascular function. The enzyme components belong to the same chymotrypsin clan of serine proteases. Advancement in molecular enzymology and functional genomics brought to light a so-far unpredicted complexity of the vascular KK-kinin system and challenged the concept that the enzymatic component simply operates by accelerating a spontaneous biochemical reaction.

The important role for the KK-kinin system in hereditary angioedema (HAE) was revealed when it was found that fluids from blisters that were induced in HAE patients contained large amounts of KK, whereas similar blister fluids obtained from control individuals did not.<sup>6</sup> It is now established that inadequate control of the KK-kinin system activation and subsequent kinin overproduction give rise to HAE.<sup>2,4,7</sup> The diagnosis is classically based on the clinical picture in combination with the reduced C1 inhibitor (C1INH) function (<50%) associated or not with low levels of C1INH and C4.<sup>8</sup> Independent observations reported HAE-nC1INH manifesting as recurrent AE<sup>9</sup> without quantitative or functional C1INH abnormalities.<sup>10,11</sup> This form may be due to mutations in the *F12* gene (without thrombosis),<sup>12,13</sup> but in most cases the genetic basis remains to be determined. The authors previously demonstrated that in HAE-nC1INH the plasma of patients transiently develops an important increase of amidase activity (using the peptide substrate, PFR-pNA), which was initially qualified as a gain-of-function of factor XII (FXII).<sup>12,14</sup> The absence of a mutation in the *F12* gene in many patients raises the hypothesis that HAE-nC1INH actually is a heterogeneous condition.<sup>15</sup> HAE-nC1INH is currently diagnosed based on the observation of manifestations reminiscent of HAE and positive family history. The disease may often be underdiagnosed or overdiagnosed because of its nonspecific phenotype as well as variable severity and penetrance.

Evidence for KK-kinin system involvement in HAE-nC1INH comes from ex vivo studies that demonstrated its activation in plasma of patients,<sup>12,15</sup> and evidence for BK formation comes from successful treatment with a B<sub>2</sub>-receptor antagonist.<sup>16</sup> Because circulating kinins are short-lived peptides ( $27 \pm 10$  s) and circulate in very low concentrations (15–90 pM during attacks), however, evidence for the increased kinin production has indirectly been demonstrated by the cleavage of HK during the attacks.<sup>17</sup>

The burden of HAE-nC1INH and the efforts to properly diagnose the disease and to develop targeted treatment are substantial.<sup>15</sup> All the kinin-forming components are present in the plasma under the proenzyme state. The activation of kinin-forming proenzymes is initiated by FXII in a reaction involving HK and plasma prekallikrein (pKK) and under the C1INH control, collectively referred to as the plasma contact system.<sup>18</sup> The triggering mechanism of this pathway and the proenzyme activation process remain, however, poorly understood.

## KININ-FORMING SYSTEM: COMPONENTS AND ACTIVATION PROCESS

The kinin-forming system is recent on the evolutionary scale: although a short BK containing protein is identified in jawless fish, the contact phase system (pKK and *F12* genes, together with a multidomain *HK* gene) appeared in some amniotes. All vertebrates and many nonvertebrate animals and arthropods, however, have a kinin-generating apparatus. This is consistent with the highly conserved development of inflammation and innate immunity between species. Moreover, it is remarkable that vascular endothelial and other cell types, including neutrophils and mast cells, express

some or all members of the protein armamentarium with which to generate, degrade, and respond to endogenous kinins both in an autocrine and paracrine fashion. Given the short half-life of circulating kinins (a few seconds), it is not surprising that they are locally generated by injured/triggered tissues and that their physiologic and patho-physiologic actions are likely to be mediated on a local level.

On endothelial cells, HK assembles on a multiprotein receptor that consists of at least gC1qR, urokinase plasminogen activator receptor (u-PAR), and cytokeratin-1 (**Fig. 1**). Moreover, CK-1 colocalizes on endothelial cells with u-PAR but not gC1qR. HK is found primarily in plasma and some tissues. The majority of plasma pKK circulates in a complex with HK, a property shared with FXII. This allows HK bound to its multiprotein receptor to serve as the pKK receptor on endothelial cells.<sup>19</sup>

### **Components of the Kinin-forming System**

In contrast to the kinin catabolism enzymes, which are spontaneously active in the plasma, the enzyme components of contact phase are present in the plasma as zymogens, which undergo enzyme conversion on activation conditions (discussed later). Terminology is summarized in **Table 1**.

#### **High-molecular-weight kininogen**

In mammalian species, there are 2 kininogens of different sizes, HK and low-molecular-weight kininogen (LK).<sup>20</sup> Synthesized in the liver, HK and LK are prototypes of modular plasma proteins that derive from differential RNA splicing of the single *KNG1* gene (11 exons).<sup>21</sup> They share the first N-terminal domains (D1–D3) and the BK D4 domain (see **Fig. 1A**). **Fig. 1B** shows the functional domains of HK interacting with its strategic partners: the N-terminal D1–D3 domains are homologous to cystatins but only the last 2 (D2 and D3) consist of specific sequences, QVVAG, that inhibit cysteine-proteases. D3 has platelet and endothelial cell-binding activity.<sup>22</sup> Adjacent to D3 is the KK-sensitive BK sequence (D4). At the C-terminal side of the BK moiety of HK, there are 2 unique domains (HK D5 and HK D6, the HK light chain [L chain]) that mediate contact phase activation.<sup>21</sup> Docking of HK to endothelium cell surface is stabilized by interactions between the His-rich sequence in the HK D5 domain and negatively charged sulfated proteoglycans on the cell surface<sup>23</sup> as well as by binding with CK-1.<sup>24</sup> With its pKK and factor XI-binding sites, HK D6 is responsible for the cofactor activity of HK in the plasma contact phase activation.<sup>25,26</sup>

HK circulates in plasma as an approximately 110-kDa single-chain glycoprotein at a concentration of 70 µg/mL to 90 µg/mL.<sup>27</sup>

#### **Factor XII**

FXII is an extraordinary protein because it links the KK-kinin system with the intrinsic coagulation cascade.<sup>28</sup> FXII is a serine protease produced by the liver that circulates in the blood as a single-chain under the zymogen form. The promoter of the *F12* gene has been shown to contain a functional estrogen-response element.<sup>29</sup> FXII consists of several structural domains: a fibronectin types II and I domain, 2 epidermal growth factor like (EGF-like) domains, a kringle domain, and a proline-rich region adjacent to its catalytic domain (**Fig. 2**). These domains are homologous to those found in other serine proteases, except the proline-rich region, which is unique to FXII.<sup>30</sup> FXII is highly susceptible to autoactivation after contact to negatively charged surfaces (eg, dextran sulfate, endotoxin, and heparin; **Table 2**) to become activated FXII (FXIIa) after a conformational change.<sup>31</sup> FXII can be also activated by KK: KK formation reciprocally activates more FXII in a reaction that is at least 1000-fold faster than autoactivation, with a reciprocal activation loop enhancing the rate of FXIIa and KK generation.



**Table 1**  
**Components of the kallikrein-kinin system**

| Current Name                           | Synonym                   | Abbreviation                    | Gene              | Plasma Concentration |
|----------------------------------------|---------------------------|---------------------------------|-------------------|----------------------|
| <b>Kinin-forming proteases</b>         |                           |                                 |                   |                      |
| Factor XII                             | Hageman factor            | FXII                            | <i>F12</i>        | 30–35 mg/L           |
| Plasma prekallikrein                   | Fletcher factor           | pKK                             | <i>KLKB1</i>      | 35–50 mg/L           |
| Prolylcarboxypeptidase                 | —                         | PCP                             | —                 | —                    |
| Tissue kallikreins                     | —                         | tKK                             | <i>KLK1-15</i>    | —                    |
| <b>Kininogens</b>                      |                           |                                 |                   |                      |
| High-molecular-weight kininogen        | Fitzgerald trait (HK) and | HK                              | <i>KNG1</i>       | 70–90 mg/L           |
| Low-molecular-weight kininogen         | Flaugeac trait (HK + LK)  | LK                              | <i>KNG1</i>       | 170–220 mg/L         |
| <b>Kinins</b>                          |                           |                                 |                   |                      |
| Bradykinin                             | —                         | BK                              | —                 | —                    |
| <i>desArg</i> <sup>9</sup> -bradykinin | —                         | <i>desArg</i> <sup>9</sup> -BK  | —                 | —                    |
| Lys-bradykinin                         | Kallidin                  | Lys-BK, KD                      | —                 | —                    |
| <i>desArg</i> <sup>10</sup> -kallidin  | —                         | <i>desArg</i> <sup>10</sup> -KD | —                 | —                    |
| <b>Kallikrein inhibitors</b>           |                           |                                 |                   |                      |
| C1 Inhibitor                           | —                         | C1INH                           | <i>SERPING1</i>   | —                    |
| $\alpha_2$ -Macroglobulin              | —                         | $\alpha_2$ -M                   | <i>A2M</i>        | —                    |
| Kallistatin                            | —                         | —                               | <i>SERPINA4</i>   | —                    |
| <b>Kininases</b>                       |                           |                                 |                   |                      |
| Angiotensin I-converting enzyme        | Kininase II               | ACE                             | <i>ACE</i>        | —                    |
| Carboxypeptidase N                     | Kininase I                | CPN                             | <i>CPN1, CPN2</i> | —                    |
| Carboxypeptidase M                     | —                         | CPM                             | <i>CPM</i>        | —                    |
| Aminopeptidase P                       | —                         | APP                             | <i>XPNPEP2</i>    | —                    |
| Dipeptidylpeptidase IV                 | CD26                      | DPPIV                           | <i>DPP4</i>       | —                    |
| Neutral endopeptidase 24.11            | CD10                      | NEP, CALLA, CD10                | <i>MME</i>        | —                    |
| <b>Accessory participants</b>          |                           |                                 |                   |                      |
| u-Plasminogen activator receptor       | —                         | u-PAR                           | <i>PLAUR</i>      | —                    |
| Plasminogen                            | —                         | —                               | <i>PLG</i>        | —                    |
| C1 proteases                           | —                         | C1r, C1s                        | <i>C1R, C1S</i>   | —                    |
| Prolylcarboxypeptidase                 | —                         | PCP                             | <i>PRCP</i>       | —                    |
| <b>Kinin receptors</b>                 |                           |                                 |                   |                      |
| B <sub>1</sub> -receptor               | —                         | B1R                             | <i>BDKRB1</i>     | —                    |
| B <sub>2</sub> -receptor               | —                         | B2R                             | <i>BDKRB2</i>     | —                    |

Activation of FXII results in cleavage of the Arg<sub>353</sub>-Val<sub>354</sub> bond, generating an heavy chain (H chain) (353 residues) and an L chain (243 residues), held together by a disulfide bond. The H chain is responsible for binding to negatively charged surfaces, involving the positively charged amino acid sequence within residues 39–47 in the fibronectin type II domain (see [Fig. 2](#)); the L chain contains the catalytic domain. FXIIa can then activate pKK, C1 complex proenzymes (C1r and C1s), and FXI. FXIIa is not able to directly cleave HK to produce BK. The FXIIa-driven contact system has proinflammatory and procoagulant activities via the KK-kinin system and the contact activation coagulation factor XI (FXI) pathway, respectively.

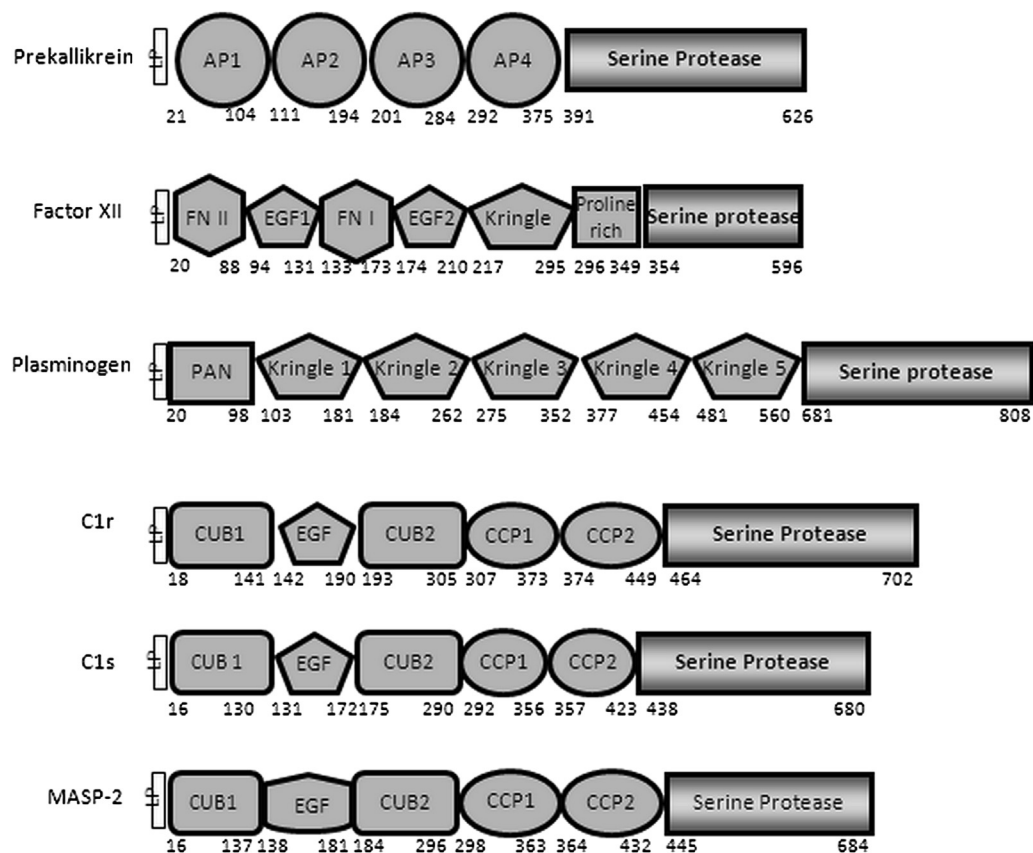


**Fig. 2.** Modular architecture of domains of pKk, FXII, plasminogen, C1r, C1s, and MASP-2. The 6 enzymes belong to the same chymotrypsin clan of serine proteases. The highly homologous catalytic domains of the serine proteases (SP domain) are located at the C-terminus. The N-terminal H chains comprise different modules specific to each protease: pKk with 4 AP modules; FXII with 2 FN modules, 2 EGF modules, a Kringle module, and an electronegatively charged poly-Pro module; plasminogen with an N-terminal PAN module and 5 Kringle modules; complement C1r, C1s, and MASP-2 with an N-terminal CUB module, a  $\text{Ca}^{2+}$ -binding EGF-like module, a second CUB module, 2 CCP modules, and a chymotrypsin-like SP domain (nomenclature and symbols are as defined in Bork and Bairoch<sup>91</sup>). AP, Apple; CCP, Sushi (SCP); EGF, EGF-like; FN I/II, fibronectin type I or type II; LP, leader peptide; MASP, MBL Associated Serine Protease.

Table 2

Contact phase activators and their effects on inflammation and angioedema

| Compound                                            | In vitro Effect<br>on Contact Phase | In vivo Effect  |            |
|-----------------------------------------------------|-------------------------------------|-----------------|------------|
|                                                     |                                     | Proinflammatory | Angioedema |
| Nonphysiologic materials                            |                                     |                 |            |
| Glass beads                                         | +                                   | —               | —          |
| Kaolin                                              | +                                   | —               | —          |
| Ellagic acid                                        | +                                   | —               | —          |
| High-molecular-weight dextran sulfate               | +                                   | —               | +          |
| OSCS                                                | +                                   | —               | +          |
| Poly I:C                                            | +                                   | —               | —          |
| Carragheenane                                       | +                                   | +               | +          |
| Biomaterials                                        | +                                   | —               | —          |
| Dialysis membranes                                  |                                     |                 |            |
| Cuprophane/polyacrylonitrile                        | +                                   | +               | +          |
| Vascular grafts<br>(Dacron/Polytetrafluoroethylene) | +                                   | +               | —          |
| Metals                                              |                                     |                 |            |
| Therapeutic compounds                               | +                                   | ?               | ?          |
| Aptamers                                            | +                                   | ?               | ?          |
| Iron oxide nanoparticles                            | —                                   | ?               | ?          |
| Endogenous substances                               |                                     |                 |            |
| Glycosaminoglycans                                  | +                                   | ?               | ?          |
| Nucleic acids (RNA, DNA)                            | +                                   | ?               | ?          |
| Sulfatide (Gal sulfate sphingolipid)                | +                                   | +               | ?          |
| Urate crystals                                      | +                                   | +               | +          |
| Misfolded protein aggregates                        | +                                   | +               | ?          |
| Amyloid-β peptide                                   | +                                   | +               | ?          |
| Polyphosphates (60–100 residues)                    | +                                   | +               | +          |
| Heparins                                            | +                                   | +               | +          |
| Chondroitin sulfate E                               | +                                   | +               | +          |
| Bacterial lipopolysaccharide                        | +                                   | +               | +          |

Data from Maas C, Oschatz C, Renné T. The plasma contact system 2.0. *Semin Thromb Hemost* 2011;37:375–81.

### Prekallikrein, plasma kallikrein, and tissue kallikreins

Two types of kallikreins (KKs) are responsible for the in vivo liberation of vasoactive kinins. These enzymes are genetically and structurally unrelated, but they both release kinins: (1) pKK is the zymogen of plasma KK, with HK as its main substrate, and is produced by the liver; (2) tissue KKs are a constitutively activated serine protease system (KK1 may be dominant), with LK as its main substrate, and originates from many cells (epithelial and endothelial cells, exocrine glandular cells, and neurons).

The pKK-encoding gene (*KLK1*) has significant homology to the gene coding for factor XI.<sup>32</sup> pKK binds to the C-terminal domain of HK (HK D6) and, through kininogen, the complex is bound on the surface of the vascular endothelium with high-affinity binding (Kd 15 nM) (Fig. 3). pKK is activated in KK during the contact phase<sup>31,33</sup>: FXIIa cleaves pKK at the scissile bond Arg<sub>371</sub>-Ile<sub>372</sub>, with subsequent production of the active KK with an H chain (371 residues) and an L chain (248 residues). The latter carries the catalytic domain (see Fig. 2).

Tissue KKs belong to a gene family comprising 15 genes (*KLK1*, the tissue KK1-encoding gene to *KLK15*).<sup>34,35</sup> KK1 is known to cleave efficiently LK, releasing

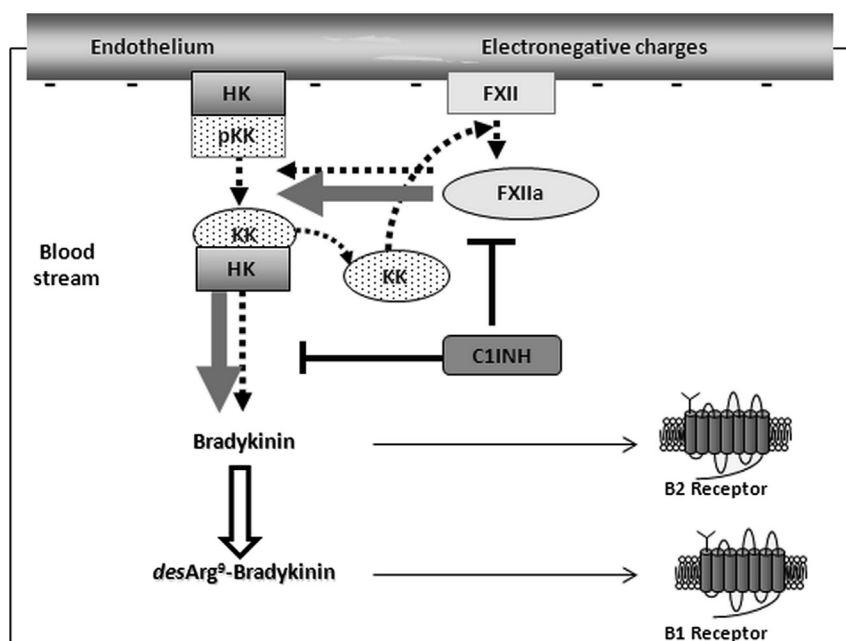


**Fig. 3.** Contact phase activation and BK production. The KK-kinin system consists of plasma proteins that assemble when blood comes into contact with triggers (eg, negatively charged surfaces): FXII, plasma pKK, and the nonenzymatic cofactor HK. Blood HK-pKK association (80%) is bound onto the endothelium. FXII and HK directly bind to polyanions, with subsequent putative conformational change in FXII leading to limited proteolytic activity. Consecutively, FXIIa cleaves pKK generating plasma KK. In an amplified reaction (*solid arrow*), KK efficiently activates further FXII zymogens and cleaves its cofactor HK to yield BK, the B<sub>2</sub>-receptor ligand. Proenzyme activation and protease activities are under the C1INH control. HSP90 and PCP activate the pKK to KK conversion (*not shown*). Coagulation factor XI, the other substrate of FXII, has no role in BK formation and is not indicated in this scheme. The circulating carboxypeptidase N (CPN) converts BK into desArg<sup>9</sup>-BK, the B<sub>1</sub>-receptor ligand, and the membrane-bound (glycosyl-phosphatidylinositol anchor) aminopeptidase P (APP) and angiotensin I-converting enzyme (ACE) inactivate the receptor-binding capacity of both peptides. (Data from Defendi F, Charignon D, Csopaki F, et al. Actualités biologiques sur les angioedèmes à kinines. Rev Francoph Lab 2012;2012:39–52.)

Lys-BK, and to have a low affinity to HK. The natural substrates of the other tissue Ks are not well established. Tissue KK can probably produce kinins in the circulation near its secretion site and influence local blood flow before it becomes inactivated.

With cleavage by a KK, HK generates BK and LK generates Lys-BK (or kallidin). Although both BK and Lys-BK assume to activate the B<sub>2</sub>-receptor with subsequent vasopermeation, the plasma KK with BK-generating capacity reflects on the major blood kinin-forming activity and is considered to mainly support the kininogenase activity responsible of the AE attacks.

### Control by the serpins

Four serpins fully control the KK-kinin system activation and activity: C1INH,  $\alpha_2$ -macroglobulin ( $\alpha_2$ -M),  $\alpha_2$ -antiplasmin ( $\alpha_2$ -AP), and antithrombin III (ATIII), in order of importance. **Table 3** shows the relative serine protease control activities and predicts the relative effectiveness of the 4 main serpins. The control capacity of KK-kinin system is

**Table 3**  
Control of contact phase, fibrinolysis, and complement proteases by serpins

| Zymogen<br>and<br>Serp | Plasma<br>Concentration<br>(μM) | Protease        | Relative<br>Effectiveness<br>of Protease<br>Control |       | Control by Serpins Ka<br>(M <sup>-1</sup> s <sup>-1</sup> ) |                               |
|------------------------|---------------------------------|-----------------|-----------------------------------------------------|-------|-------------------------------------------------------------|-------------------------------|
|                        |                                 |                 |                                                     |       | C1<br>Inhibitor                                             | α <sub>2</sub> -Macroglobulin |
| FXII                   | 0.37                            | FXIIa           | C1INH                                               | 91.3% | 7.4 × 10 <sup>4</sup>                                       | —                             |
|                        |                                 |                 | α <sub>2</sub> -AP                                  | 3.0%  |                                                             |                               |
|                        |                                 |                 | ATIII                                               | 1.5%  |                                                             |                               |
|                        |                                 |                 | α <sub>2</sub> -M                                   | 4.3%  |                                                             |                               |
| pKK                    | 0.4                             | Kallikrein      | C1INH                                               | 52%   | 1.4 × 10 <sup>5</sup>                                       | 4.8 × 10 <sup>4</sup>         |
|                        |                                 |                 | α <sub>2</sub> -M                                   | 35%   |                                                             |                               |
|                        |                                 |                 | α <sub>2</sub> -AP                                  | 8%    |                                                             |                               |
|                        |                                 |                 | ATIII                                               | 1.1%  |                                                             |                               |
| C1s                    | 0.3                             | C1s<br>protease | C1INH                                               | 100%  | 4.3 × 10 <sup>5</sup>                                       | —                             |
| C1r                    | 0.3                             | C1r<br>protease | C1INH                                               | 100%  | 4.1 × 10 <sup>4</sup>                                       | —                             |
| MASP-2                 |                                 | MASP2           | C1INH                                               | 100%  | 1.6 × 10 <sup>5</sup><br>1.3 × 10 <sup>6</sup><br>(heparin) | —                             |
| C1INH                  | 1.7                             | —               | —                                                   | —     | —                                                           | —                             |
| α <sub>2</sub> -M      | 3.6                             | —               | —                                                   | —     | —                                                           | —                             |
| α <sub>2</sub> -AP     | 1.1                             | —               | —                                                   | —     | —                                                           | —                             |
| ATIII                  | 2.6                             | —               | —                                                   | —     | —                                                           | —                             |

Abbreviations:  $\alpha_2$ -AP,  $\alpha_2$ -antiplasmin;  $\alpha_2$ -M,  $\alpha_2$ -macroglobulin; ATIII, antithrombin III; MASP-2, MBL-associated serine protease 2.

performed under conditions of a serpin:protease molar excess (approximately 5:1) and a low binding affinity ( $k_{\text{on}}$   $1.4\text{--}41 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ). Plasma concentrations of C1INH are most effective in inactivating KK (see [Table 3](#)). Sustained activation of the contact phase and complement, which can lead to depletion of C1INH, results in reduced control of FXII and KK. This decrease allows the reciprocal activation to proceed between these 2 proteases, resulting in sustained release of BK.

Tissue KK activity is under the control of the endogenous serpin kallistatin (serpin A4), a potent but nonexclusive inhibitor present in both blood and tissues with a slow serpin-protease complex forming kinetics<sup>36</sup> and KK-independent anti-inflammatory actions.<sup>37</sup>

### Kallikrein-kinin System Activation

#### Activation process

As a largely established principle, the activation of the kinin system proceeds on electronegative surfaces. It involves a proenzyme to enzyme conversion, with getting hold of serine proteases exhibiting limited proteolytic activities to activate another zymogen and to cleave the HK substrate with subsequent BK production, so contact activation describes the unique property of FXII to undergo autoactivation and amplification with a putative change in shape when exposed to negatively charged surfaces. This assumption is strengthened by the low  $k_{\text{on}}$  values of the serine protease-serpin interactions (approximately  $10^5 \text{ M}^{-1} \text{ s}^{-1}$ ; see [Table 3](#)). The molecular basis for the formation of FXIIa remains, however, unknown. This process is likely to amplify fibrinolysis

and complement zymogens and vice versa (discussed later). Although many biologic substances allow for FXII autoactivation, their exposure to the zymogen does not occur constitutively in the intravascular compartment in nondisease states. This perception is particularly relevant for HAE-nC1INH patients where the amidase activity increases in plasma during the attack but keeps within the normal range through the interphase.<sup>17</sup>

On human umbilical vein endothelial cells, prolylcarboxypeptidase (PCP) has been identified as a pKK activator with serine protease properties and a  $K_M$  of 6.7 nM.<sup>38</sup> In addition, recent studies argue for direct effects of FXII on the protease-activated receptor (PAR3), which may contribute to contact system-driven platelet activation.

During activation of the pKK-HK complex on endothelial cells, BK is locally released from HK. FXIIa and plasmin also release kinins *in vitro*, but their importance in kinin formation *in vivo* is not established.

### **Contact phase activators**

Nonphysiologic materials were the first substances described to activate contact phase; kaolin and celite are commonly used for coagulation diagnostics. The endothelial glycosaminoglycans are natural activators of FXII with BK production at the vessel wall.<sup>23,39</sup>

Aggregates of amyloid- $\beta$  peptide initiate activation of KK-kinin system with production of BK in the cerebrospinal fluid of patients suffering from Alzheimer disease.<sup>40</sup> Platelets support FXII activation dependent on integrin  $\alpha$ IIb- $\beta$ 3 signaling and enhance activation of the KK-kinin system in plasma.<sup>41,42</sup> On activation, platelets release from their dense granules various substances, including polyphosphates consisting of an inorganic linear polymer of phosphate units. Recent studies identified the platelet-derived polyphosphate responsible for FXII activation as a polymer of 60 to 100 phosphate residues. Platelet polyphosphate activates FXII in reconstituted systems and in plasma via direct binding, with subsequent FXIIa-dependent BK formation *in vitro* and *in vivo*.<sup>43</sup> High-molecular-weight dextran sulfate (500 kDa), a polysulfated polysaccharide of glucose moieties, is a potent stimulator of contact phase. Once injected in animals, it induces transient systemic BK-mediated hypotension; the drop of blood pressure is blocked by icatibant.<sup>44</sup> In 2007, lethal acute anaphylactoid hypotension affected patients receiving intravenous commercial heparin. A contaminant was identified in suspect preparations of heparin and characterized as non-natural occurring oversulfated chondroitin sulfate (OSCS).<sup>45</sup> OSCS activates contact phase and complement as potentially as dextran sulfate and triggers KK-mediated BK formation in human plasma and in a model of experimental hypotonic shock *in vivo*.<sup>46</sup> Physiologic analogs (eg, mast cell chondroitin sulfates) have been proposed as activators of the contact phase *in vitro*.<sup>47</sup> The potency to contact-activate FXII decreases from dextran sulfate and OSCS (4 sulfate residues per disaccharide) to heparin (2.7 sulfate residues). Despite that the exact mechanism of proenzyme FXII activation is not known, BK-forming activity seems dependent on negative charge density of the polysaccharide rather than on a defined structure. **Table 2** gives an overview of the activators of contact phase.

Kinins are released under conditions of ischemia. Demonstration of increased kinin levels and possible protective effects of kinins during myocardial ischemia in the absence of ACE inhibitors support the contention that kinins may play a role in myocardial ischemia: in isolated normoxic rat hearts, kinins are released into the perfusate.<sup>48</sup> During ischemia, the respective kinin outflow increased more than 5-fold; thus, ischemia is a stimulus for an enhanced kinin release with contribution to reduce the sequelae of myocardial ischemia.<sup>49</sup>

In bacterial infection, bacterial endotoxins are released and can activate zymogens of the contact phase. Kinins are then produced in the systemic circulation and participate in septic shock.<sup>50</sup>

The KK-kinin system can also be activated by contact with artificial surface in extracorporeal circulations with subsequent kinin information and hypotension.<sup>51</sup>

### **Endothelium**

The autoactivation process of FXII can include negatively charged surfaces (see [Table 2](#)). This activation along the endothelium requires zinc-dependent binding of FXII and HK to gC1qR, CK-1, and u-PAR. These 3 proteins complex together onto the cell membrane and the initiation depends on autoactivation of FXII upon binding to gC1qR. Activation of the contact system can also proceed on endothelial cell in the absence of FXII: in this instance, pKK activation depends on interaction with heat shock protein 90 (Hsp90)<sup>52</sup> or PCP.<sup>38</sup>

### **Neutrophil and mast cell**

KK is a chemotactic factor for neutrophils<sup>53</sup>; it has been shown to cause neutrophil aggregation<sup>54</sup> and release of elastase.<sup>55</sup> FXIIa has also been shown to stimulate neutrophil aggregation and degranulation.<sup>56</sup> Neutrophils express B<sub>1</sub>-receptor and the agonist stimulation of this receptor induces the neutrophil recruitment during inflammatory response<sup>57</sup>; desArg<sup>9</sup>-BK, the B<sub>1</sub>-receptor (B1R) ligand, increases endothelial production of chemokines, thereby facilitating the B1R-mediated neutrophil extravasation.<sup>58</sup> HK binding to neutrophils has been shown to depend on zinc concentration and MAC-1 (CD11b/CD18).<sup>59</sup>

Activated neutrophils and mast cells can secrete proteases with KK-like activity (eg, elastase that enables the formation of kinins,<sup>60</sup> tryptase that also could release kinins from both HK and LK in addition to activate pKK,<sup>61</sup> and neutrophil that secretes tissue KK).<sup>62</sup> In addition to histamine, mast cell secretory granules also contain highly sulfated polysaccharides with heparin as a major constituent. This glycosaminoglycan is synthesized exclusively by mast cells.<sup>63</sup> It has been identified as an FXII contact activator in vitro<sup>47</sup> and can initiate in vivo BK formation in an FXII-dependent manner.<sup>64</sup> This is consistent with the participation of the mast cell component in the BK-dependent vascular permeability.

## **INTERPLAY BETWEEN CONTACT PHASE, FIBRINOLYSIS, AND COMPLEMENT**

Complement and the kinin system are closely linked in the endothelial permeability phenomenon.

In observation of AE patients, a production of plasmin is observed during the acute attacks.<sup>65</sup> FXII and KK activate plasminogen in plasma,<sup>66</sup> resulting in plasmin formation. Plasminogen activation by FXII may be due to the high homology of FXII and tissue plasminogen activator (tPA), a fibrinolysis initiator.<sup>30</sup> On cell surfaces, plasminogen activation is triggered by BK-stimulated release of tPA from endothelial cells.<sup>67</sup> u-PAR or urinary-type plasminogen activator can also activate plasminogen and can be of primary importance for its cell-mediated activation. In addition, the activation of fibrinolysis enhances kinin release by KK in inflammatory situations due to collaborative actions of plasmin and KK on HK.<sup>68</sup> The favorable effect of prophylactic treatment with tranexamic acid in patients with HAE (whatever the type of HAE) indicates that fibrinolysis activation could take place in BK production.<sup>69</sup> Tranexamic acid (4-[amino-methyl]cyclohexanecarboxylic acid) is a synthetic lysine derivative that forms a reversible complex with plasminogen at the lysine binding site. The reduction in plasminogen

binding to fibrin seems to result in a decrease in the production of t-PA by endothelial cells or an increase in the rate of its clearance.

The contact phase has also the capacity to activate the complement classical<sup>70,71</sup> and alternative convertases.<sup>72</sup> Early work has shown that the rabbit ortholog of plasma KK is able to release the C5a as a result of a limited proteolysis of C5.<sup>73</sup> FXIIa has been demonstrated to activate complement C1 and subsequently the classical convertase.<sup>71</sup> In vitro, FXIIa triggers activation of the classical convertase and initiates the fibrinolysis via PK-mediated urokinase activation. On mast cells, C5a causes an upregulation of plasminogen activator inhibitor-1, which is able to neutralize the enzymatic activity of tPA.<sup>74</sup> Simultaneous activation of contact phase and complement often occurs in several pathologic conditions, including triggering conditions of AE. The actual control of these multiple protease systems by C1INH is also indicative of the close relationship between these proteolytic systems (see [Table 3](#)).<sup>75</sup> Evidence for interaction between complement and contact system is also supported by in vitro demonstration (using the Transwell model system) that the cytolytically inactive terminal complement complex induces the vascular leakage by the formation of BK and the release of platelet-activating factor.<sup>76</sup> It has also been shown that gC1qR, the receptor for the globular heads of C1q, specifically binds the HK L chain.<sup>77</sup> The molecular complex formed by gC1qR, u-PAR and CK-1 on the endothelial surface is able to bind pKK through HK and also binds FXII.

**Fig. 4** shows the interconnections between the 3 proteolytic cascades.

## PATIENT INVESTIGATIONS: GENETICS, BIOLOGIC PHENOTYPE, AND TESTING

HAE-nC1INH patients were formerly grouped under the name, idiopathic nonhistaminergic AE; this included both hereditary and sporadic forms; patients suffering from AE not prevented by anti-H1-histamine whose etiopathogenesis remains undefined.<sup>78</sup> The first familial observations reported clinical features indistinguishable from those of patients presenting with C1INH deficiency.<sup>9,11,79</sup> Frequently their AE was prevented by prophylaxis by tranexamic acid, a nonspecific antifibrinolytic drug likely to limit the amplification pathway dependent on plasmin.<sup>69</sup>

**Table 4** shows the biologic characteristics of patients presenting with HAE-nC1INH. The plasma spontaneous amidase activity was assayed using the peptide substrate, H-D-Pro-Phe-Arg-pNA. The severity of disease depends on the kinin catabolism, mainly supported by aminopeptidase P, angiotensin-I converting enzyme, carboxypeptidase N, and dipeptidylpeptidase IV.

Some light has been shed on etiopathogenic mechanisms that may underlay substantially idiopathic AE. Starting from evidence for linkage of the disease status with genetic markers on chromosome 5q in German and French kindreds, mutational analysis revealed a Thr328Lys mutation in the positional candidate gene *F12*.<sup>12</sup> In several other families, missense or deletion mutations in the *F12* gene segregated with the symptoms.<sup>13,64,80–82</sup> The Thr328Lys mutant was defined as a gain-of-function enzyme although it is still unexplained why the catalytic serine protease domain of the protease is untouched by the mutation. The additional electropositive charge in the polypyrroline domain of FXII could increase the susceptibility of proenzyme to activation; thus, gain-of-activation might be a more precise definition of the functional effect conferred by the FXII<sup>Lys328</sup> protein. As an additional hypothesis, an escape of the mutant proenzyme to the control by C1INH could provide the FXII<sup>Lys328</sup> protein with a functional benefit in the enzyme activation. A large majority of HAE-nC1INH situations are not associated with this mutant expression, and some patients exhibit a similar biologic phenotype, in favor of another protein mutant and in line of the observation of familial pedigrees.

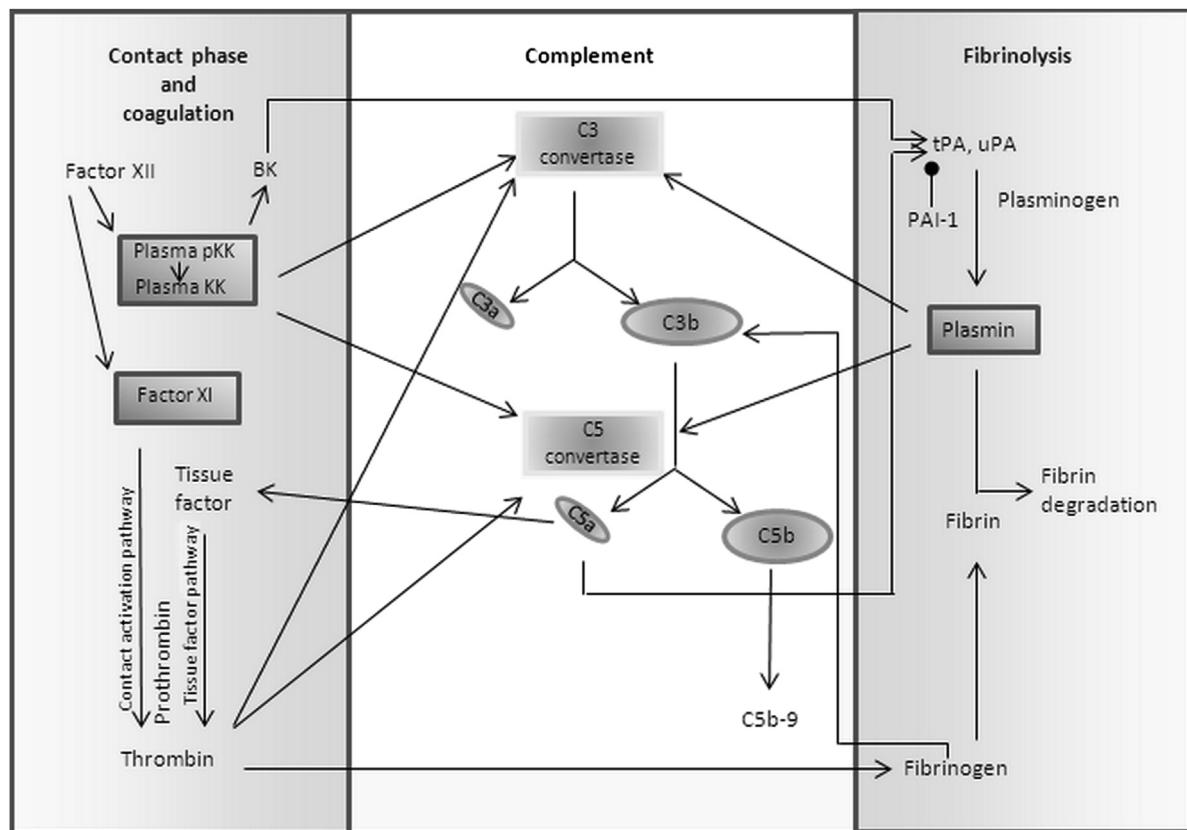


**Fig. 4.** Interplay between contact phase and complement. Components of the contact phase, fibrinolysis, and complement cascades develop positive interactions.

**Table 4**  
**Interpretation of patient data**

| Angioedema Type               | Spontaneous Amidase Activity | Kinin Catabolism Enzymes                                                                                                                                       |
|-------------------------------|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| HAE-nC1Inh-F12c               | ↑ to ↑↑↑ <sup>a</sup>        | —                                                                                                                                                              |
| HAE-nC1Inh-F12nc <sup>b</sup> | ↑ to ↑↑↑ <sup>a</sup>        | —                                                                                                                                                              |
|                               | —                            | ↓ APP (OMIM <sup>c</sup> 300145)<br>or<br>↓ ACE (OMIM <sup>c</sup> 106180)<br>or<br>↓ CPN (OMIM 212070 and 603103)<br>or<br>↓ DPPIV (OMIM <sup>c</sup> 102720) |

Biologic investigation of HAE-nC1INH carrying or not the *F12* mutation (F12c and F12nc, respectively) is based on the measurements of plasma spontaneous amidase activity and the kinin catabolism.

*Abbreviations:* ACE, angiotensin I-converting enzyme; APP, aminopeptidase P; c, carrier; CPN, carboxypeptidase N; DPPIV, dipeptidylpeptidase IV; nc, noncarrier.

<sup>a</sup> During the AE attack.

<sup>b</sup> With the only biologic distinction by the C1INH function, failed kinin catabolism conditions and exaggerated kinin-forming activities overlap in the HAE-nC1INH entity.

<sup>c</sup> Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/omim>).

In some instances, the cleavage of C1INH was observed during the active periods of disease.<sup>14</sup> The cleavage was sustained during the pregnancy and C1INH function was found progressively decreased during pregnancy to nearly 50% at delivery, with important C1INH serpin cleavage without serpin-protease association.<sup>64</sup> The C1INH function returns to normal value out of the active period of disease. As a hypothesis, AE occurs in a context of uncontrolled proteolysis of C1INH serpin subsequent to the abundant contact phase proteases after activation of the Hageman factor mutant.

When patients present with an attack, plasma HK is transformed into the separated H and L chains, with or without the remnant native HK protein, as displayed by immunoblot with an anti-L-chain antibody.<sup>83</sup> The BK release during the attack can be appreciated from the abundance of the native HK compared with controls and the observation of the L-chain 46-kDa species gives evidence of the high protease input during the attacks (Bertrand Favier, DVM, PhD, personal communication, 2013). Complementary to HK analysis, the area under the curve of the kinetics of BK and desArg<sup>9</sup>-BK production in plasma on in vitro contact phase activation gives a more precise estimation of the kinin production during the attack.<sup>84</sup>

## REPORTING, FOLLOW-UP, AND CLINICAL IMPLICATIONS

The absence of modified parameters commonly used in HAE diagnostic renders difficult the biologic diagnostic of HAE-nC1INH. In the absence of the identification of an *F12* gene mutation, the short-term increase of the plasma amidase activity in the periods of attacks is informative of the development of contact phase proteases and probably associated HK cleavage with BK production.<sup>17</sup> This transient kinin production in the AE condition is consistent with early observations where plasma, obtained from patients during an attack, strikingly increases vascular permeability in guinea pig skin, but samples obtained between attacks lack this property.<sup>85</sup>

As described previously, the HAE-nC1INH condition is commonly observed in female individuals. Because women were affected by this type of AE, the first descriptions referred the disease to as estrogen-dependent AE. Symptoms are actually

precipitated by high estrogen levels, whether endogenous (pregnancy) or exogenous (oral contraceptives, hormone replacement therapy, or both) in origin.<sup>11</sup> This feature has been postulated to be associated with the estrogen-response element in the 5' flanking region of the *F12* gene.<sup>29</sup> The level of plasma FXII was found normal, however, in affected female individuals. This condition of high AE frequency in female individuals coincides with the higher reference interval of the spontaneous amidase activity of female blood donors compared with that found in the male population (3.5 ratio).<sup>17</sup> The higher susceptibility of women and the triggering property of the estrogen input, however, remain unexplained.

Because of the rarity of the *F12* gene mutation within the families with HAE-nC1INH, the plasma amidase activity is useful for biologic testing.<sup>14,86</sup> It has been evaluated in patients presenting with HAE-nC1INH compared with HAE with C1INH deficiency, acquired angioedema, histamine-dependent AE, and anaphylaxis reactions. By fulfilling the conditions of the sampling in the period of attack, this assay may assist physicians and patients with meaningful argument. In complement to this assay, the evaluation of the HK cleavage and the kinetic parameters of the in vitro kinin production give evidence of the BK dependency of AE and an estimation of the BK production during the last hours.

## OUTCOMES

**Table 4** describes the distribution of AE etiopathologies in excessive kinin-production (including HAE with C1INH deficiency) and failed kinin catabolism. HAE-nC1INH can be recognized with these 2 conditions. Identification of families with failure of the kinin catabolism will contribute to distinguish between subgroups the HAE-nC1INH entity in the next future. In addition, independent etiopathogenesis conditions with both abnormalities of kinin formation and kinin catabolism can concur in situations related to HAE-nC1INH.<sup>87</sup> **Fig. 4** describes the activation of C3 convertases by KK, with subsequent anaphylatoxin production in line with the probable involvement in mast cell activation and the development of urticaria associated with HAE-nC1INH.<sup>88</sup>

## CURRENT CONTROVERSIES/FUTURE CONSIDERATIONS

HAE-nC1INH seems to be a heterogeneous disease. As far as is known, mutations in the *F12* gene have been described as causative for some families with HAE-nC1INH and the presence of the SNP -2399A in the *XPNPEP2* gene as a possible aggravating factor.<sup>81</sup> In addition, low activity of plasma metallopeptidases of the kinin catabolism in HAE-nC1INH situations may give arguments for a responsibility of kininases in HAE-nC1INH. This leaves room for other gene mutations (in a monogenic sense) that could be involved in the development of HAE-nC1INH. Apart from this, the differences found in expressivity of the disease within families (also in families with *F12* mutations) leads the authors to postulate that the phenotype may be influenced by modifier genes.<sup>82</sup>

The question remains whether a proportion of disease cases might be multifactorial in origin. It could be speculated that inflammatory processes associated with kinins are related to infection conditions, where hosts can in principle use 2 different strategies to defend themselves against parasites: resistance and tolerance. Animals typically exhibit considerable genetic variation for resistance (ie, the ability to limit pathogen burden), when little is known about whether animals can evolve tolerance (ie, the ability to limit the damage caused by a given pathogen burden).<sup>89,90</sup> Considering the hypothesis that a certain proportion of AE cases might represent a multifactorial disease etiology, the pathologic load (ie, pathogen burden in the Råberg and his colleagues

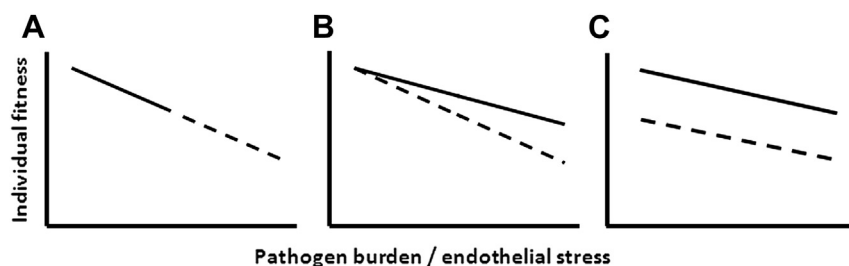


**Fig. 5.** Simplified scheme showing reaction norms of 2 individual genotypes (*dotted or solid line*) for disease severity across a range of pathologic burden in individuals. (A) Two equally tolerant genotypes differing in resistance; (B) two equally resistant genotypes (same average endothelial stress), but the genotype described with the dotted line is less tolerant; and (C) the genotypes differ in neither resistance (same average endothelial stress) nor tolerance (same slope). (Data from Råberg L, Sim D, Read AF. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 2007;318:812–4.)

illustration) can be extended to endothelial stress as pathologic burden. Individuals suffering from AE might be distinguished between their capacities to develop resistance or tolerance to the AE trigger by 3 schemes (Fig. 5). In Fig. 5A, the genotype with the dotted line has lower endothelial stress (is more resistant) and thereby maintains a higher health status. In Fig. 5B, the genotype with the dotted line is less tolerant (health declines faster with increasing endothelial stress). In Fig. 5C, the gene difference exists even when the individuals are unstressed.

## SUMMARY

Contact phase proenzymes are activated in conditions when endothelium is triggered with diverse materials and under various conditions. The proenzyme to enzyme conversion is under control by serpins, and proteolytic activity develops toward HK. HAE-nC1INH is associated with this biologic condition and, in a few cases, a mutation in the *F12* gene. Endothelium, mast cells, fibrinolysis, and complement participate in the triggering and the amplification of the process.

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