
Hereditary angioedema: a current state-of-the-art review, III: mechanisms of hereditary angioedema

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Objective: To review the available evidence on the pathophysiologic mechanism of episodes of edema in hereditary angioedema (HAE).

Data Sources: MEDLINE and PubMed were searched using the following keywords: *hereditary angioedema*, *C1 inhibitor*, *complement system*, *contact system*, and *bradykinin*.

Study Selection: Studies were selected based on their relevance to the pathophysiologic features of HAE.

Results: Early studies from the 1970s and 1980s disagreed as to whether the symptoms in HAE were mediated via complement or contact system activation. Studies have demonstrated that, in vitro, in C1 inhibitor (C1-INH)-deficient plasma, only contact system activation results in generation of a vascular permeability enhancing factor. Furthermore, individuals who express a variant C1-INH that is a normal inhibitor of contact system proteases but is deficient in the ability to inactivate complement system proteases do not develop angioedema. The blood of patients with HAE, during attacks, contains elevated levels of cleaved high-molecular-weight kininogen and bradykinin. Last, C1-INH-deficient mice develop increased vascular permeability that is mediated via contact system activation.

Conclusions: Hereditary angioedema attacks are mediated by bradykinin generated via contact system activation. The specific factors that trigger attacks remain unclear.

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INTRODUCTION

Although hereditary angioedema (HAE) was described earlier, its hereditary nature was first clearly recognized in 1888 by Sir William Osler,¹ who also provided the first complete, detailed, clinical description of this autosomal-dominant inherited disease. The explanation for this inheritance pattern is that people with HAE are heterozygous for deficiency of C1 inhibitor (C1-INH). Donaldson and Evans² were the first to recognize that C1-INH was deficient in the plasma of patients with HAE. Complete deficiency of C1-INH in humans has not been observed. Simple absence of expression from one allele, with resulting decreased expression of C1-INH in the plasma, is referred to as type 1 HAE, whereas expression of a dysfunctional C1-INH protein and decreased levels of normal protein is type 2 HAE.

The mechanism of edema formation in HAE has remained somewhat controversial until relatively recently. The controversy, in a sense, began with the initial observation by Landerman and colleagues³ that the plasma of patients with HAE was deficient in plasma kallikrein inhibitory capacity. When

Donaldson and Evans² showed that deficiency of C1-INH resulted in HAE, the only known function of C1-INH, inactivation of C1, had been described only 6 years earlier.⁴ Later studies demonstrated that C1-INH inactivates C1r and C1s, the protease subcomponents of C1.⁵ The role of C1-INH in regulation of contact system activation, via inactivation of plasma kallikrein and factor XIIa, was accomplished during the 1970s and 1980s.⁵ Because C1-INH regulated both systems, the question became, “Which system is responsible for the generation of symptoms?” Over the next 3 decades, a number of studies were published that supported either complement or contact system mediation. The accumulated data strongly indicate that the primary (and likely the only) mediator of symptoms is bradykinin. However, the specific biochemical triggers that initiate angioedema remain ill defined.

THE BIOLOGICAL FUNCTION OF C1-INH

C1-INH is the only serine protease inhibitor that plays a significant role in regulation of complement activation. It is the primary regulator of classic pathway activation via inactivation of C1r and C1s (Table 1).⁵ It also regulates lectin pathway activation via inactivation of a C1s-like protease known as mannan-binding lectin-associated protease 2 (MASP2), which is activated after binding of mannan-binding lectin to a microorganism surface.⁶ α_2 -Macroglobulin also is able to inactivate MASP2.⁶ However, the relative contributions of C1-INH and α_2 -macroglobulin toward lectin pathway regulation have not been determined. C1 inhibitor also

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Table 1. The Contribution of C1-INH to Protease Inhibition in Plasma

Protease	% of plasma inhibitory capacity provided by C1-INH
Complement system	
C1r	100
C1s	100
MASP2	^a
Contact system	
Plasma kallikrein	42–84
Coagulation factor XIa	47
Coagulation factor XIIa	90
Fibrinolytic system	
Tissue plasminogen activator	^b
Plasmin	^b

Abbreviations: C1-INH, C1 inhibitor; MASP2, mannan-binding lectin-associated protease.

^a Undetermined, probably the primary inhibitor; α_2 -macroglobulin may play some role.

^b C1-INH plays little, if any, role under normal circumstances, but small amounts of complexes with tissue plasminogen activator and plasmin may be observed in some situations (angioedema attacks, endotoxin shock, and exhaustive exercise).

inhibits alternative pathway activation by reversibly binding to C3b, which inhibits factor B binding.⁶ This activity differs from inhibition of the classic and lectin pathways in that it does not involve protease inhibition. However, the potential in vivo importance of this activity remains to be determined.

C1-INH is responsible for most of the inhibition of plasma kallikrein and coagulation factor XIIa and is, therefore, the major regulator of contact system activation (Table 1).^{5,6} α_2 -Macroglobulin also contributes to inactivation of these pathways.^{5,6} C1-INH also inactivates factor XIa and, therefore, is also involved in regulation of the intrinsic coagulation pathway. In vitro, C1-INH also inactivates several other proteases, in particular plasmin and tissue plasminogen activator (tPA).^{5,6} Plasmin, in vivo, is inhibited primarily by α_2 -antiplasmin; C1-INH does not play a major role in its regulation.^{5,6} C1-INH may participate, to some degree, in tPA inactivation.⁷ Under some circumstances, C1-INH may play a minor role in the regulation of coagulation and fibrinolysis. For example, coagulation and fibrinolytic pathway activation have been shown during HAE attacks.^{8,9} It is not clear whether this indicates that C1-INH is normally involved in regulation of these pathways or if activation of coagulation and fibrinolysis may induce attacks of angioedema. In addition, small amounts of complexes with tPA and plasmin are present in plasma in endotoxin shock and during exhaustive exercise.

PATHOPHYSIOLOGIC CHARACTERISTICS OF ANGIOEDEMA ATTACKS

Attacks of angioedema in HAE are not associated with signs of inflammation, although attacks may be triggered by in-

flammatory conditions. There also is no indication that allergy plays a role. Although some reports initially suggested that histamine levels in the urine were elevated during HAE attacks, a later study¹⁰ demonstrated that urinary histamine levels are not elevated. Furthermore, antihistamines are ineffective. Epinephrine also is ineffective in HAE.

C1-INH is a member of the serpin family of serine protease inhibitors. Serpins inactivate proteases by exposing a peptide within a surface loop that mimics the natural substrate of the protease. This sequence is recognized and cleaved by the protease, but the result, rather than release of the protease, is formation of a covalent serpin-protease complex. Therefore, the protease and the serpin are inactivated. This inactivation of the inhibitor via complex formation may be a factor in triggering episodes of angioedema. Activation of any of the several proteases that may be inactivated by C1-INH would result in inactivation and consumption of the inhibitor. If the rate of consumption exceeded the rate of synthesis, the C1-INH plasma level would decrease, which, if substantial, could result in an episode of angioedema. This consumption would be amplified if activation of 1 system induced activation of either or both of the other 2 systems (Fig 1). In vitro, factor XIIa and plasmin may activate C1, and factor XIIa or kallikrein may generate plasmin from plasminogen, but the in vivo relevance of these observations remains unclear.^{11–17} Angioedema sometimes develops during therapy with recombinant tPA,^{18,19} which suggests that plasmin may activate the contact system. The data suggest that this angioedema is mediated by bradykinin.^{19,20} Trauma or inflammation, which commonly induce symptoms in HAE, might result in the

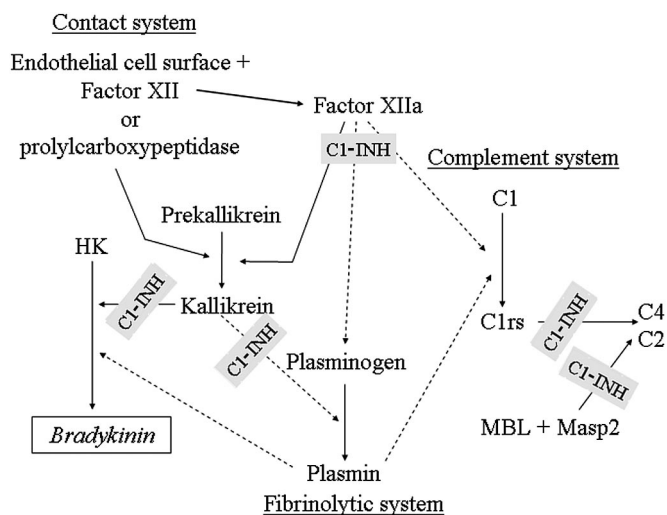


Figure 1. Potential interactions among the complement, contact, and fibrinolytic systems. Solid lines with arrows indicate reactions of defined biological relevance, and dotted lines with arrows indicate reactions that have been demonstrated in vitro but that are of unproven biological importance. C1-INH indicates C1 inhibitor; HK, high-molecular-weight kininogen; MASP2, mannan-binding lectin-associated protease 2; MBL, mannan-binding lectin.

generation of sufficient plasmin to induce contact system activation. This would be consistent with the clinical effectiveness of plasmin inhibitors in HAE.

MEDIATION OF SYMPTOMS IN ANGIOEDEMA

The Complement and Contact Systems in HAE Attacks

Both the complement and contact systems are activated during HAE attacks. Low plasma levels of C1-INH in HAE result in apparent spontaneous activation of the complement and contact systems. This clearly occurs during attacks of angioedema but may also take place at a low level during asymptomatic intervals. The most readily identifiable indication of contact system activation is the presence of circulating, cleaved, high-molecular-weight kininogen during episodes of angioedema.²¹ C2 and C4 levels are decreased during an attack and also may be low when asymptomatic. The catabolic rate of C1-INH in symptom-free patients with HAE is elevated,²² which probably largely accounts for the fact that plasma levels of C1-INH are much less than 50% of normal, as would be expected with heterozygous deficiency. Circulating activated C1²³ and C1-INH C1r and C1s complexes have been detected.²⁴

Evidence for a Complement System–Generated Mediator

Because the complement and contact systems are activated during attacks, for a number of years it was not clear which system was responsible for the mediation of symptoms. Beginning in the late 1960s, a number of studies suggested that symptoms were mediated via complement system activation, whereas others suggested contact system mediation. The argument for a complement-derived mediator was based on the clinical observation that angioedema is nonpainful, whereas subcutaneous injection of bradykinin, which also produces edema, is quite painful. Donaldson and colleagues²⁵ showed that plasma obtained from patients with HAE during symptom-free periods, after incubation at 37°C, generated a factor that contracted smooth muscle and had vascular permeability-enhancing activity when injected intradermally into guinea pig skin. The data suggested that the kininlike activity was a product of complement system activation: C2 and C4 seemed to be required for its generation.^{25–27} The factor responsible for the activity seemed to be distinct from bradykinin in its size, electrophoretic mobility, isoelectric point, and susceptibility to trypsin. In addition, its generation seemed to be inhibited by antibodies to C2 and C4.

Other data indirectly supported the studies indicating that the mediator was complement derived. Intradermal injection of C1s in guinea pigs and humans resulted in local swelling without pain or itching.^{28,29} C2-deficient humans had no response to intradermal C1s, but a C3-deficient patient did respond.^{28,30} C2-deficient guinea pigs were unresponsive but did respond after intravenous infusion of C2.³¹ These data were consistent with the previously described data in that they suggest that a mediator of edema requires C2. More important, however, none of these data provided clear-cut

direct evidence that the complement-derived mediator was the same as the mediator that caused angioedema in HAE.

Other data suggested that the complement-derived kininlike factor might result from plasmin cleavage of C2 during complement activation. Incubation of C1s, C2, and plasmin at 37°C, whether in the presence or absence of C4, generated enhanced vascular permeability and smooth muscle–contracting activity.^{32,33} Furthermore, synthetic peptides identical to the carboxyl terminal sequence of C2b possessed vascular permeability and smooth muscle–contracting activity.³² However, although these synthetic peptides enhanced vascular permeability, their specific activity was low compared with bradykinin. Another study³⁴ questioned these results and was unable to detect any kininlike activity or cleavage of C2 or C2b after incubation with plasmin.

Evidence for a Contact System–Generated Mediator (Bradykinin)

In 1980, Curd and colleagues³⁵ demonstrated that fluid from induced blisters from patients with HAE contained large amounts of kallikrein compared with that of control individuals. These data suggested that the contact system in patients with HAE was more readily activated than in people without the disease. Another study³⁶ demonstrated decreased levels of prekallikrein and high-molecular-weight kininogen in patients with HAE during attacks. These studies, although they provided clear-cut evidence for contact system activation, did not provide proof that angioedema was mediated via the contact system rather than the complement system. In addition, 2 studies^{37,38} in the early 1980s provided evidence for bradykinin generation during attacks of angioedema. Curd et al³⁷ and Fields et al³⁸ reported that incubation of HAE plasma in the presence of EDTA, which prevents complement system activation, resulted in the generation of a smooth muscle–contracting activity that was resistant to heat and trypsin but was sensitive to carboxypeptidase B. In addition, Curd et al³⁷ showed that the activity in HAE plasma cochromatographed with bradykinin on high-performance liquid chromatography. Unfortunately, these findings were reported only in abstract form.³⁷ Then, in 1986, Berrettini and colleagues,²¹ as previously noted, demonstrated cleaved high-molecular-weight kininogen in the plasma from a patient with HAE during an attack. Subsequently, this group and others^{39–42} have confirmed this finding in additional patients.

By the late 1980s, the derivation of the mediator of angioedema in HAE remained controversial: as previously outlined, some data suggested a complement system origin (ie, a C2 kinin), whereas other data indicated that the mediator was bradykinin. In 1994, Shoemaker et al⁴³ sought to begin to clarify this issue by repeating, as closely as possible, the experiments described originally by Donaldson et al.²⁵ Both HAE plasma and “artificial HAE plasma,” prepared by immunoabsorption of normal or other deficient plasma with anti-C1-INH antibody, were incubated at 37°C in attempts to generate vascular permeability-enhancing factors. Vascular permeability-enhancing activity was generated from HAE

plasma and from normal plasma depleted of C1-INH. Furthermore, this factor was elicited from C1-INH-depleted C2-deficient plasma, but not from plasma deficient in prekallikrein, factor XII, or high-molecular-weight kininogen. This factor was isolated and sequenced: it was bradykinin.⁴³ In 1998, Nussberger et al⁴⁴ demonstrated increased bradykinin levels in plasma from patients with HAE during angioedema attacks. These 2 groups of observations did not show a direct cause-and-effect relationship between bradykinin and angioedema. However, they did suggest that bradykinin may be involved in the mediation of angioedema.

A family originally described by Wisniewski et al⁴⁵ provided further insight into the pathogenesis of HAE. Several members of this kindred had extremely low C4 levels combined with a dysfunctional C1-INH molecule, as defined by its inability to normally inhibit C1. This deficiency in inhibition of C1 apparently resulted in enhanced C4 consumption. The family was discovered because one member presented with systemic lupus erythematosus. Each of the 10 family members with low C4 levels expressed a C1-INH molecule that had lost approximately 90% of its ability to inhibit C1r and C1s but retained perfectly normal inhibition of plasma kallikrein and coagulation factor XIIa.^{46,47} This dysfunction was the result of a point mutation adjacent to the reactive center arginine that resulted in substitution of an alanine with a valine (Ala443Val).⁴⁶ No family member with this mutation, all of whom had ongoing complement system activation, had ever experienced an episode of angioedema. This observation makes it highly unlikely that classic complement system activation alone results in a biologically important mediator of angioedema.

Another approach toward resolving this issue was to develop an animal model for C1-INH deficiency. The C1-INH knockout mice were derived using randomly targeted embryonic stem cell lines obtained from Lexicon Genetics (The Woodlands, Texas).⁴⁸ The C1-INH-deficient mice, heterozygous (C1-INH^{+/-}) and homozygous (C1-INH^{-/-}), seemed normal and grew and reproduced normally. The C1-INH^{-/-} mice expressed neither C1-INH messenger RNA nor protein. In C1-INH^{+/-} mice, C1-INH plasma levels were 50% of normal or less. C4 levels were also decreased but were not as low as in most humans with HAE. This, combined with the observation that the mice have not developed peripheral angioedema, indicates that the consequences of C1-INH deficiency in the mouse are less severe than in humans. Eight C1-INH^{-/-} mice did have episodes of gastrointestinal edema, and one of these was accompanied by laryngeal edema.

Although no wild-type littermates or any other mice in the facility were affected, there were no recurrences, the triggering event was not identified, and attempts to induce attacks were unsuccessful. Therefore, it cannot be stated with certainty that these episodes were a result of C1-INH deficiency.

Although C1-INH-deficient mice do not have any obvious abnormalities, intravenous injection of Evans blue dye, which binds to serum albumin, revealed increased vascular permeability compared with wild-type littermate controls. Shortly after injection, the skin of the feet and around the nose and eyes in C1-INH^{-/-} and C1-INH^{+/-} mice turned blue, whereas the skin of C1-INH^{+/+} mice became only slightly blue.⁴⁸ Spectrophotometric measurement of the extracted dye showed that the permeability of the deficient mice was approximately 1.5 times that of the wild-type mice. Intravenous supplementation with C1-INH (100 µg) before infusion of Evans blue dye reversed the increased vascular permeability. Therefore, the defect clearly was a result of the C1-INH deficiency.

By performing appropriate crosses, C1-INH bradykinin 2 receptor (Bk2R) double-deficient mice were generated. Not only did these mice seem normal, but they also were indistinguishable from wild-type littermates in their vascular permeability response. Therefore, this receptor is necessary to mediate the increased vascular permeability response. Because bradykinin is the only known ligand for the Bk2R, these results suggest that bradykinin is the mediator of the increased vascular permeability. These results were strengthened by treating C1-INH^{-/-} mice with kallikrein inhibitors and with a Bk2R antagonist (Table 2). The protease inhibitors used were DX-88 (Dyax Corp, Cambridge, Massachusetts), a recombinant-engineered Kunitz domain protease inhibitor, and a recombinant variant C1-INH with the same mutation (Ala443Val) as the family previously described. DX-88 is a potent highly specific inhibitor of plasma kallikrein, whereas the C1-INH variant inhibits plasma kallikrein and factor XIIa but is a poor inhibitor of C1r and C1s.^{46,47,49} In addition, mice were treated with a bradykinin receptor antagonist, Icatibant (formerly known as HOE140) (Jerini AG, Berlin, Germany), which is a decapeptide antagonist with a structure similar to that of bradykinin. Treatment with each of these agents abrogated the increased vascular permeability.

CONCLUSIONS

The major biological roles of C1-INH relate to the regulation of vascular permeability and the modulation of inflammation. The C1-INH regulates activation of the complement and

Table 2. C1-INH-Deficient Mice: Modulation of Increased Vascular Permeability

Agent	Mechanism	Permeability	Interpretation
DX-88	Kallikrein inhibition	Decreased	Decreased bradykinin generation
C1-INH Ala443Val	Kallikrein inhibition	Decreased	Decreased bradykinin generation
Icatibant	Bk2R antagonism	Decreased	Decreased signaling via Bk2R
Captopril	ACE inhibition	Increased	Decreased bradykinin degradation

Abbreviations: ACE, angiotensin-converting enzyme; Bk2R, bradykinin 2 receptor; C1-INH, C1 inhibitor.

contact systems. Suppression of contact system activation by inactivation of plasma kallikrein and factor XIIa, which prevents bradykinin release, helps to maintain the endothelial barrier. The loss of this function is illustrated by the recurrent episodes of angioedema that develop in patients with HAE. As outlined in this review, the conclusion that bradykinin is the primary mediator of angioedema is based on several independent lines of evidence, including in vitro studies, analysis of patients, and analysis of C1-INH-deficient mice. The C1-INH-mediated regulation of complement system activation results primarily in anti-inflammatory effects. In addition, however, as discussed previously, consumption of C1-INH as a result of protease inhibition after complement system activation may play a role in the induction of attacks of angioedema.

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