

Mechanism of Angioedema in First Complement Component Inhibitor Deficiency

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Characterization of the pathophysiology of hereditary angioedema (HAE) began in 1962 with the observation by Landerman and colleagues [1] that plasma of patients who had hereditary angioedema was deficient in plasma kallikrein inhibitory capacity and the subsequent demonstration by Donaldson and Evans [2] that the deficient protein was first complement component (C1) inhibitor. The role of C1 inhibitor in regulation of complement system activation had been described shortly after the first description of the isolation and characterization of the first complement component; it was characterized as a heat labile factor in plasma that inhibited the esterolytic activity of C1 [3]. C1 was shown to consist of three separate proteins, C1q, C1r, and C1s, the last two of which are zymogen serine proteases that are converted to their proteolytically active forms following binding of C1q to an immune complex. Subsequent studies demonstrated that C1 inhibitor inactivates both C1r and C1s [4,5].

The role of C1 inhibitor in regulation of the contact system, by means of inactivation of both plasma kallikrein and factor XIIa, was elucidated during the 1970s and 1980s [6–14]. C1 inhibitor also is capable of inactivating a number of other proteases, including plasmin and tissue plasminogen activator (tPA) [15–19]. The evidence clearly indicates that C1 inhibitor is not a major regulator of plasmin, which, in vivo, is inhibited primarily by α_2 antiplasmin [20,21]. Some data, however, suggest that C1 inhibitor may participate in tPA inactivation [16]. Cugno and colleagues [22,23] have demonstrated activation of both the coagulation cascade and the fibrinolytic

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pathway during attacks of HAE. Therefore, C1 inhibitor may be involved in regulation of these pathways *in vivo* or, alternatively, activation of these pathways by exogenous factors may trigger attacks of angioedema.

One might have expected that the demonstration of the specific deficiency responsible for HAE and characterization of the proteases inactivated by C1 inhibitor would lead rapidly to characterization of the mechanism of generation of angioedema symptoms. However, largely because C1 inhibitor is the primary regulator of activation of both the classic pathway of complement and the contact system of kinin generation, the mechanism remained unclear until recently. At this point, the accumulated information clearly indicates that the primary, and most likely the sole, mediator of symptoms is bradykinin generated by means of activation of the contact system. However, the specific biochemical events leading to the initiation of an angioedema attack remain ill defined.

Function of first complement component inhibitor

C1 inhibitor is a member of the serpins, a family of proteins consisting mostly, but not solely, of serine protease inhibitors. All members of the serpin family share similar sequences and three-dimensional structures and consist of seven to nine α helices and three β sheets. The most distinctive features of the native serpin structure are a five-stranded β sheet (sheet A) that makes up the prominent planar surface of the molecule and a peptide loop (the reactive center loop) consisting of approximately 17 amino acid residues that is exposed at one pole of the molecule. The reactive center loop is located near the carboxyl terminus of the protein. The native serpin structure is rather unstable and is sensitive to denaturing conditions. Cleavage by a nontarget protease within the reactive center loop results in a dramatic molecular rearrangement, with insertion of this loop into β sheet A as its fourth strand, converting sheet A to a six-stranded sheet. The cleaved inhibitor is a much more stable structure that may be demonstrated by enhanced resistance to thermal or chemical denaturation [24–26]. This rearrangement is indicated by the expression of neopeptides on the cleaved molecule [27–29].

Serpins inactivate proteases after recognition by the protease of a substrate-like sequence located within the reactive center loop. The protease attacks the peptide bond carboxyl terminal to the P1 residue, which, in the case of C1 inhibitor, is an arginine. The inhibitor is cleaved at this site. However, rather than subsequent release of the protease, a covalent bond is formed between the reactive center amino acid residue of the serpin and the active site serine of the protease [30]. Subsequently, the reactive center loop inserts into β sheet A, as with cleavage by nontarget proteases. This insertion moves the protease to the opposite pole of the molecule. The result is a stable cleaved serpin structure covalently linked to a destabilized protease in which its catalytic triad is interrupted. Both the protease and the inhibitor are thereby inactivated, an aspect that led to the designation of serpins as

suicide substrates [31]. The structural and functional data all indicate that the mechanism of inactivation of proteases by C1 inhibitor is the same as the mechanism with other serpins [31,32].

The most significant difference between C1 inhibitor and other serpins is in the size and characteristics of its nonserpin amino terminal domain. This domain in C1 inhibitor is quite large (approximately 100 residues) and is heavily glycosylated with three N-linked and at least seven O-linked carbohydrates [33]. Some portions of the sequence of this domain are mucin-like. Based on functional studies using a recombinant C1 inhibitor molecule with the amino terminal 100 residues deleted, this domain plays no role in protease inhibitor function [34]. Recent studies have indicated that the amino terminal domain is required, however, for a direct interaction with gram-negative bacteria and endotoxins [35,36]. This interaction may play a role in C1 inhibitor-mediated protection from sepsis and septic shock.

C1 inhibitor is the only protease inhibitor that inactivates C1r and C1s [4,5] and is, therefore, the primary regulator of classic pathway activation (Fig. 1). It also plays a role in regulation of the lectin pathway, which is activated by interaction of mannan-binding lectin or ficolins with surface structures of a variety of micro-organisms [37–39]. C1 inhibitor inactivates MASP2, a C1s-like protease that is associated with mannan binding lectin and ficolins and is activated following binding. However, in vivo, MASP2 also most likely is inactivated by α_2 macroglobulin [40]. The relative importance of each is not yet clear. Another complement regulatory function of C1 inhibitor has been described, in which C1 inhibitor was shown to bind to C3b, which resulted in inhibition of factor B binding, similar to the mechanism of action of factor H [41]. This function was independent of protease inhibitor activity. Although the resulting alternative pathway inhibition was shown to take place at physiologic concentrations in vitro, the importance of this activity in vivo remains unclear.

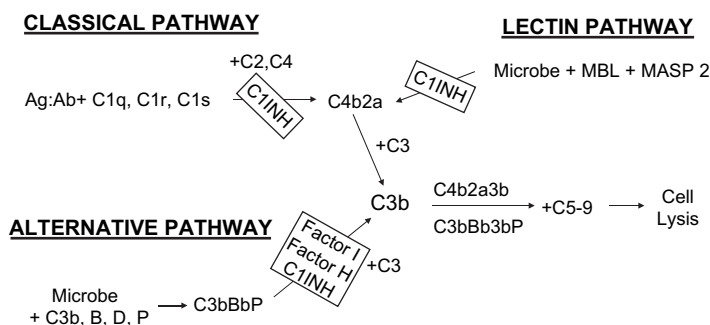


Fig. 1. Complement system activation. The components enclosed within rectangles are inhibitors of activation. Ag:Ab, antigen-antibody complexes; B, factor B; C1INH, C1 inhibitor; D, factor D; MASP 2, Mannan-binding lectin associated protease 2; MBL, mannan-binding lectin; P, properdin.

C1 inhibitor is the primary inhibitor of both plasma kallikrein and coagulation factor XIIa, although these proteases can also be inactivated by α_2 macroglobulin [6,9,12–14]. It is therefore the major regulator of contact system-mediated bradykinin generation. By virtue of its inactivation of both factor XIIa and factor XIa, C1 inhibitor also inhibits activation of the intrinsic coagulation pathway. The contact system is activated by a variety of exogenous negatively charged surfaces, such as glass, kaolin, elagic acid, dextran sulfate, and possibly endotoxin lipopolysaccharide [42–47]. Factor XII binds to these surfaces; this binding induces its autoactivation to proteolytically active factor XIIa, which activates both plasma prekallikrein and additional factor XII (Fig. 2). Activated kallikrein then cleaves two peptide bonds in high molecular weight kininogen to release bradykinin. A biologically relevant negatively charged substance responsible for activation in vivo has not been described. Activation in vivo most likely takes place primarily on the surface of endothelial cells [48–53].

At least two pathways that lead to activation of kallikrein in the absence of factor XII have been described. One of these is reported to be mediated by an interaction between the kallikrein–high molecular weight kininogen complex and heat shock protein 90 [50,54,55], whereas the other depends on activation of prekallikrein by prolylcarboxypeptidase (see Fig. 2) [56–59]. Some data suggest that the prolylcarboxypeptidase-mediated activation mechanism is quite efficient and may be the primary mechanism of contact system activation in vivo [56,59]. By contrast, other data suggest that both this and heat shock protein 90–mediated activation are quite slow, but that they are greatly accelerated in the presence of factor XII [50,54,55]. Although the details of contact system activation remain to be resolved, it is

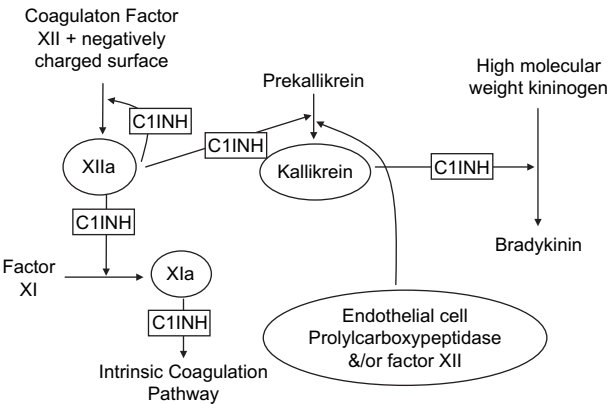


Fig. 2. Contact system activation. The diagram indicates the two pathways of contact system activation, one by means of exogenous negatively charged surfaces and the other taking place on the surface of endothelial cells. Activation of prekallikrein on endothelial cells is proposed to be mediated by prolylcarboxypeptidase or by factor XII. C1INH, C1 inhibitor.

clear that the primary mechanism of regulation of activation of the contact system is inhibition of kallikrein (and factor XIIa) by C1 inhibitor.

Mutations resulting in first complement component inhibitor deficiency

Individuals who have HAE are heterozygous for mutations that result either in lack of expression of C1 inhibitor protein or in the synthesis of a non-functional protein from one allele together with expression of normal protein from the other allele. Clinically, these two types are referred to as type 1 and type 2, respectively, based on whether a dysfunctional protein is detectable in the patient's plasma. However, this division is not precisely accurate. A number of mutations result in a dysfunctional protein that is either degraded intracellularly, secreted inefficiently, or cleared rapidly from the circulation. Strictly speaking, these should be referred to as type 2, but, because they are either absent or present in very small quantities in the blood, they are usually classified as type 1. In clinical practice, a patient is classified as type 2 based on the finding of a normal (or elevated) C1 inhibitor level determined immunochemically, together with a decreased functional level of C1 inhibitor. Using these criteria, approximately 80% to 85% of patients are classified as type 1.

Mutations resulting in deficiency may be of virtually any type, including deletions (and duplications) that range in size from a few base pairs to multiple exons and a variety of single base substitutions. Large deletions and single base changes at the reactive center are somewhat overrepresented in comparison with the other types of mutations. All of the large mutations appear to be a consequence of recombination involving the multiple Alu repetitive elements that are present within the introns of the gene. The reactive center mutations most likely are a result of the cytosine-guanine (CG) dinucleotide within the codon for the reactive center arginine (CGC). The CG dinucleotide is susceptible to mutation due to deamidation of methylated cytosine, which results in conversion to a thymine. This process may occur in the CG dinucleotide in the coding strand or in the complementary strand. With C1 inhibitor, this results in substitution of the reactive center arginine with either cysteine or histidine. These make up the largest single group of mutations within the C1 inhibitor gene. However, it should be noted that some other mechanism may also be at play here, because several serine and leucine substitutions at the reactive center have been described, which cannot result from the deamidation mechanism.

Whether a patient is type 1 or type 2 is of no known clinical importance. No differences exist between the two in clinical presentation, severity, or clinical course. Furthermore, with two possible exceptions, the specific mutation does not appear to have any clinical effect. These potential exceptions are one family with a large deletion, in which the abnormal transcript appears to inhibit transcription of the normal gene, and another family with a small duplication, which appears to result in inhibition of translation of

the normal C1 inhibitor transcript [60,61]. It is not known whether these mechanisms apply in other families with different mutations.

Pathophysiologic interpretation of clinical characteristics

Patients who have HAE develop recurrent acute episodes of localized edema that may involve the skin, the mucosa of the gastrointestinal tract, the pharynx, or the larynx. Therefore, patients who have C1 inhibitor deficiency have an intermittent defect in the regulation of vascular permeability. This increased vascular permeability results from a sudden local loss of endothelial barrier function within the postcapillary venule. Signs or symptoms of inflammation are absent, as are any signs of an allergic component. Although early reports suggested that urinary histamine levels were elevated during attacks of angioedema, antihistamines are ineffective, and a study of a number of patients who had HAE (both symptomatic and asymptomatic) demonstrated that urinary histamine levels are not elevated [62]. The angioedema in HAE does not respond to treatment with epinephrine.

Serpins are suicide substrates, which may be one important factor in the initiation of attacks of angioedema. Activation of any protease inhibited by C1 inhibitor, whether a complement, contact, or fibrinolytic system protease, will result in inactivation and consumption of the inhibitor. If the rate of consumption exceeds the rate of ongoing synthesis, the C1 inhibitor plasma level will decrease. C1 inhibitor levels in patients who have HAE average approximately 30% of normal during symptom-free periods. Significant reduction below this level is associated with the development of symptoms. Therefore, any event that triggers activation of any of the three proteolytic pathways would increase C1 inhibitor consumption and suppress the plasma level and could result in an episode of angioedema. Clearly, trauma and inflammation, which are known initiators of attacks of angioedema, can result in activation of each pathway. However, not all episodes of angioedema are associated with an obvious precipitating factor. In addition, consumption of C1 inhibitor could be amplified if activation of one system directly resulted in activation of either or both of the other two systems (Fig. 3). Activated factor XII and plasmin may activate C1, and factor XIIa or kallikrein may generate plasmin from plasminogen, but the biologic significance of these findings has not been clearly demonstrated [63–69].

Another possibility with some clinical support is that plasmin generation might activate the contact system, with resulting bradykinin generation, and might thus result in the development of angioedema. Angioedema sometimes develops during therapy with recombinant tissue plasminogen activator [70,71]. Experimental evidence suggests that this angioedema is mediated by bradykinin released by the plasmin that is generated by the infused tissue plasminogen activator [71,72]. It is possible that, in a patient who has HAE, activation of the fibrinolytic pathway during trauma or inflammation might generate sufficient plasmin for a similar phenomenon to occur.

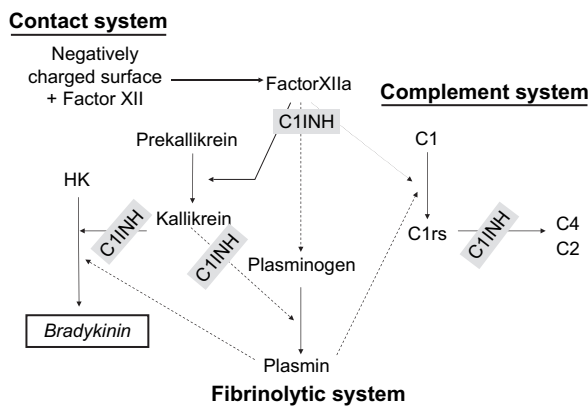


Fig. 3. Proposed interactions among the complement, contact, and fibrinolytic systems. Arrows with solid lines indicate reactions of known biologic relevance. Arrows with dotted lines indicate reactions demonstrated to take place in vitro, but that are of uncertain in vivo importance. C1INH, C1 inhibitor; HK, high molecular weight kininogen.

This hypothesis is consistent with the clinical observation that plasmin inhibitors are frequently effective in HAE.

The mediation of angioedema

Complement system activation

In HAE, the low plasma levels of C1 inhibitor result in apparent spontaneous activation of both the complement and contact systems. Although this is most obvious during an overt attack of angioedema, it is highly likely that activation occurs to some degree during symptom-free periods. C2 and C4 levels, which are virtually always low during an attack, may also be low when the patient is asymptomatic [73–75]. Another indication of activation is the observation that the catabolic rate of C1 inhibitor in symptom-free HAE patients is elevated in comparison with that of normal individuals [76]. This aspect also probably accounts for the finding that plasma levels of C1 inhibitor are much less than 50% of normal, which would be expected in a heterozygous deficiency state [77]. During periods of angioedema, circulating activated C1 [73,78] and complexes of C1 inhibitor with both C1r and C1s may be detected [79], in addition to decreased plasma levels of C2 and C4, which may be so low as to be undetectable.

During the 1970s and 1980s, a number of studies were published that suggested that the mediator of angioedema was a product of complement system activation. On a clinical basis, a complement-derived mediator appeared most likely to many investigators because angioedema is nonpainful, whereas subcutaneous injection of bradykinin, which also produces edema, is quite painful. Donaldson and colleagues [80] showed that plasma taken from

HAE patients during symptom-free periods, following incubation at 37°C, generated a factor that contracted smooth muscle and had vascular permeability-enhancing activity when it was injected intradermally into guinea pig skin. The data suggested that this kinin-like activity was derived from complement activation; furthermore, both C2 and C4 appeared to be required for its generation [80–82]. Specifically, the factor appeared to differ from bradykinin in size, electrophoretic mobility, isoelectric point, and susceptibility to trypsin, and its generation was inhibited by antibodies to C2 and C4.

Various other data also appeared to support the hypothesis that the mediator was complement derived. Intradermal injection, in guinea pigs and humans, of proteolytically active C1s resulted in local swelling without pain or itching (ie, it was similar to angioedema) [83–85]. Furthermore, C2-deficient people did not respond to intradermal injection of C1s, whereas a C3-deficient patient did respond [84,86]. C2-deficient guinea pigs also were unresponsive, but the response was restored following intravenous infusion of C2 [87]. Therefore, to induce a vascular permeability response by means of complement activation, C2 was required. However, these studies did not identify the source of the mediator. In addition, there were no clearcut direct data to indicate that the mediator resulting from complement system activation was the same as the mediator responsible for angioedema in HAE.

Several studies suggested that the complement-derived factor might result from plasmin cleavage of C2 during complement activation. First, mixtures of C1s, C2, and plasmin, in the presence or absence of C4, generated vascular permeability-enhancing and smooth muscle-contracting activity [88,89]. This event appeared to coincide with the release of a group of small peptides from the carboxyl terminus of C2b [88]. In addition, several synthetic peptides matching the carboxyl terminal sequence of C2b possessed vascular permeability and smooth muscle-contracting activity [88]. The most active of these was 25 amino acids long. However, although this synthetic peptide clearly enhanced vascular permeability, its specific activity was low in comparison with bradykinin. Another study was unable to demonstrate any cleavage of C2 or C2b with plasmin, and no kinin-like activity resulted from such incubation mixtures [90].

Contact system activation

During the same period, evidence for contact system activation in HAE was also accumulating. Among the first of these findings was the demonstration that large amounts of active kallikrein were present in induced blister fluid from patients who had HAE. This finding suggested a lower threshold for contact system activation in comparison with normal individuals [91]. In addition, during attacks, patients who had HAE had decreased levels of prekallikrein and of high molecular weight kininogen [92]. At least two early studies suggested that bradykinin was generated during attacks of

angioedema [93,94]. One of these reported, unfortunately only in abstract form, that bradykinin was directly detectable in plasma from HAE patients following in vitro incubation [93]. In 1986, Berrettini and colleagues [95] demonstrated that high molecular weight kininogen was circulating in a cleaved form in a patient who had HAE. Subsequently, this group and a number of others have confirmed this finding in additional patients [96–99].

Therefore, by the end of the 1980s, it was clear that both the complement and contact systems were activated during episodes of angioedema. However, the precise mediator in HAE had not been unequivocally identified. Many investigators believed that the mediator was bradykinin, whereas others believed that a “C2 kinin” was responsible. In 1994, Shoemaker and colleagues [100] sought to begin to clarify this issue by repeating, as closely as possible, the protocol followed previously by Donaldson and colleagues [80]. For these experiments, in addition to HAE plasma, “artificial HAE plasma” was prepared by immunoabsorption with anti-C1 inhibitor antibody. This technique made possible the use of a variety of deficient plasmas, which then also were made deficient in C1 inhibitor. Vascular permeability-enhancing activity could be generated both from HAE remission plasma and from normal plasma depleted of C1 inhibitor. More importantly, the activity was also readily generated from C1 inhibitor-depleted C2-deficient plasma, but not from plasma deficient in prekallikrein, factor XII, or high molecular weight kininogen. Furthermore, the active factor was isolated and shown by amino acid sequence analysis to be bradykinin [100]. Subsequently, Nussberger and colleagues [101] demonstrated that bradykinin levels were elevated in the plasmas of patients who had HAE during episodes of angioedema. Although these data do not demonstrate a cause and effect relationship between bradykinin and angioedema, they strongly suggest that bradykinin is involved. The data also do not rule out the possibility that more than one mediator is involved.

*A dysfunctional first complement component inhibitor
with diminished inhibition of complement proteases
but normal inhibition of contact system proteases*

In 1994, Wisniewski and colleagues [102] described a kindred with extremely low C4 levels as a result of an apparent dysfunctional C1 inhibitor molecule that was defective in its capacity to inhibit C1. This defect presumably resulted in excessive C4 consumption. The propositus from this family presented with systemic lupus erythematosus. Subsequent studies demonstrated that every family member with a low C4 level expressed a C1 inhibitor molecule that was defective in its ability to inhibit both C1r and C1s but retained a perfectly normal ability to inactivate plasma kallikrein and coagulation factor XII [103–105]. This mutant C1 inhibitor retained only approximately 10% of its ability to inhibit proteolytically active C1r and C1s. This dysfunction was due to a mutation that resulted in substitution

of the alanine residue immediately amino terminal to the reactive center arginine (the P2 position, Ala443) with a valine [103]. Most importantly, neither the single patient who has systemic lupus nor any of the seven other family members who express this dysfunctional protein has ever had angioedema. These individuals clearly had ongoing complement system activation, as indicated by their depressed plasma C4 levels. Therefore, complement system activation alone does not result in the generation of any peptide that induces angioedema. This family provides strong indirect evidence that the mediator in HAE is bradykinin.

The first complement component inhibitor-deficient mouse

Analysis of C1 inhibitor knockout mice has provided additional support for the hypothesis that bradykinin is the mediator of angioedema in HAE [106]. A database of genes randomly targeted with a retroviral-mediated gene trapping technique developed by Lexicon Genetics (The Woodlands, Texas) was screened. The C1 inhibitor gene was targeted in two embryonic stem cell lines, one of which was used to develop the deficient mice. Both the C1 inhibitor heterozygous deficient (C1INH^{+/-}) and homozygous deficient (C1INH^{-/-}) mice were normal in appearance, grew and developed normally, and reproduced normally. Litter size was normal in both C1INH^{+/-} and C1INH^{-/-} matings, which suggests that there was no increase in embryonic or fetal death. Neither C1 inhibitor mRNA nor protein was detected in C1INH^{-/-} mice. Similar to observations in patients who have HAE, C1 inhibitor plasma levels in C1INH^{+/-} mice were less than 50% of normal. C4 protein levels and functional complement levels were variably decreased in most C1 inhibitor-deficient mice, although probably not to such an extent as in humans who have HAE. Therefore, the mice appear to have unregulated complement activation, although possibly not to such an extent as do patients who have HAE.

Another indication that the consequences of C1-inhibitor deficiency in the mouse are less dramatic than in the human is provided by the observation that none of the mice have ever developed angioedema involving the skin. A total of only eight deficient mice have had spontaneous episodes of gastrointestinal edema with obstruction; one of these mice also had laryngeal edema. None of the wild-type littermate mice were affected, nor were any other mice in the colony. However, the episodes have not recurred in other deficient mice. Unfortunately, the trigger for these episodes is unknown, and attempts to reproduce such episodes have not been successful. Although these events did appear to be angioedema, it is not possible definitively to ascribe them to C1 inhibitor deficiency, both because they have not been reproduced and because no biochemical data exist from the time of the attacks.

Although C1 inhibitor-deficient mice do not have any clinically apparent abnormalities, when a marker for plasma protein extravasation (Evans Blue dye) was used, the mice were shown to have increased vascular permeability

in comparison with wild-type littermate controls (C1INH^{+/+}). C1INH^{-/-}, C1INH^{+/-}, and C1INH^{+/+} mice were injected intravenously with Evans blue dye, which readily binds to serum albumin. Within minutes, the skin of the feet and the skin around the nose and eyes of both the heterozygous and homozygous deficient mice became quite blue, whereas that of the wild-type mice became only slightly blue (Fig. 4) [106]. The vascular permeability was increased to a greater extent than in the wild-type mice by the topical application of mustard oil, which enhances local inflammation. The degree of vascular permeability was quantitated spectrophotometrically by extraction of the dye from the feet of the mice. The dye extravasation both in the C1INH^{+/-} and in the C1INH^{-/-} mice was approximately 1.5 times greater than that in the wild-type mice (Table 1). Enhancement with mustard oil increased the difference to 3- to 3.5-fold greater in the deficient mice. Treatment of the deficient mice with intravenous C1 inhibitor (100 μ g) before injection with Evans blue dye completely reversed the increased vascular permeability. The amount of vascular leak in the treated deficient mice was indistinguishable from that of their wild-type littermates. This finding strongly indicates that the increase in vascular permeability in the knockout mice was solely a result of the deficiency of C1 inhibitor and not of some unexpected associated defect.

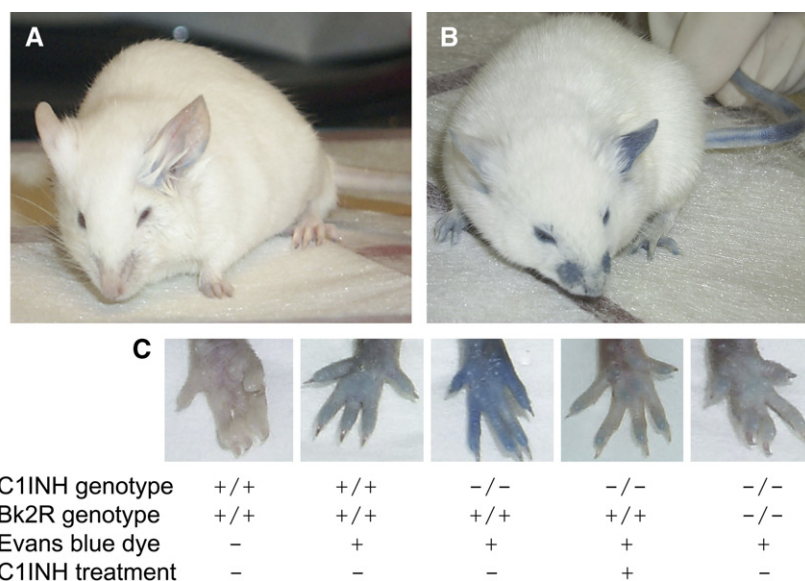


Fig. 4. Analysis of vascular permeability. Extravasation of Evans blue dye 15 to 30 minutes after injection. (A) Wild-type mouse; (B) C1 inhibitor-deficient mouse; (C) rear footpads of mice of the indicated genotypes with or without C1 inhibitor treatment. Bk2R, bradykinin 2 receptor; C1INH, C1 inhibitor.

Table 1
Vascular permeability in C1-inhibitor-deficient mice

C1INH genotype	Bk2R genotype	No. of mice	C1INH treatment	Fold change in dye extravasation vs. WT ^a
+/-	+/+	5	None	1.5*
-/-	+/+	5	None	1.6*
-/-	-/-	5	None	1.0
+/-	+/+	4	C1INH	1.1
-/-	+/+	4	C1INH	1.0
-/-	+/+	22	DX-88	1.0
-/-	+/+	25	Icatibant	0.9
-/-	+/+	11	P2 A → V	1.0

Abbreviations: Bk2R, bradykinin 2 receptor; C1INH, C1 inhibitor; P2 A → V, P2 alanine to valine; WT, wild type.

^a The dye extravasation was quantitated spectrophotometrically at 600 nm. The mean values of each group are expressed as the fold increase compared with an untreated wild-type control group.

* $P < .002$ compared with wild-type controls.

Data from Han ED, MacFarlane RC, Mulligan AN, et al. Increased vascular permeability in C1 inhibitor deficient mice is mediated by the bradykinin type 2 receptor. *J Clin Invest* 2002;109:1057–63; and Han Lee E, Pappalardo E, Scafidi J, et al. Approaches toward reversal of increased vascular permeability in C1 inhibitor deficient mice. *Immuno Letters* 2003;89:155–60.

To test the hypothesis that the increased vascular permeability was mediated by bradykinin, C1INH^{-/-} mice were crossed with bradykinin 2 receptor (Bk2R) knockout mice. The resulting C1INH^{+/-}, Bk2R^{+/-} mice then were mated, which resulted in all the expected genotypes in the predicted proportions. The C1INH^{+/-}, Bk2R^{+/-} and the C1INH^{-/-}, Bk2R^{-/-} mice all appeared normal. Furthermore, they were indistinguishable from the wild-type mice in their vascular permeability response (see Table 1). Therefore, the Bk2R is required to mediate the increased vascular permeability response. Bradykinin is the only known ligand for the Bk2R. These data strongly indicate that bradykinin is the mediator of the increased vascular permeability in the mice.

To confirm and strengthen these observations, mice also were treated with two agents that inhibit contact system activation but have little or no effect on complement system activation, and with another that is a Bk2R antagonist. The first agent, DX-88 (Dyax Corp., Cambridge, Massachusetts), is an engineered Kunitz domain protease inhibitor prepared using phage display. It is a potent and highly specific inhibitor of plasma kallikrein. The second kallikrein inhibitor used was the recombinant mutated C1 inhibitor in which the P2 Ala had been substituted with a Val [103–105], diminishing its inhibition of C1r and C1s but having no effect on its ability to inhibit plasma kallikrein. The bradykinin receptor antagonist used was Icatibant (Jerini AG, Berlin, Germany), a decapeptide antagonist with a structure similar to that of bradykinin. Each of these agents completely reversed the increased vascular permeability (see Table 1).

Because the bradykinin receptor antagonist and both inhibitors of contact system activation prevent the increased vascular permeability, it appears unlikely that any mediator other than bradykinin is involved.

Although the C1 inhibitor-deficient mice have an obvious defect in vascular permeability, they appear to have compensated quite well for this defect. Furthermore, it is surprising that the phenotype in these mice appears identical in the C1INH^{+/-} and C1INH^{-/-} mice. No human with complete deficiency of C1 inhibitor has been described, which suggests that complete deficiency might be lethal. The explanation for the relative mildness of the phenotype in the mice in comparison with humans who have angioedema is unknown, as is that for the lack of difference between the heterozygous and homozygous deficient mice. A likely potential explanation for both findings is that, in the mouse, other inhibitors play a larger role in regulation of C1 inhibitor target proteases than is the case in the human. For example, although in both the mouse and the human, C1 inhibitor appears to be the only inhibitor of activated C1r and C1s, in the human it is not the only inhibitor of factor XIIa and plasma kallikrein. Depending on the methods used, in human plasma C1 inhibitor provides from 42% to 84% of the kallikrein inhibitory capacity and as much as 90% of the inhibitory capacity toward activated factor XII [9,12–14,107]. The remainder of the plasma inhibitory activity toward both kallikrein and factor XIIa is provided by α_2 macroglobulin. It is possible that α_2 macroglobulin, or perhaps other inhibitors, provides a higher proportion of the inhibition of murine contact system proteases. This hypothesis remains uninvestigated.

Another potential explanation for the lack of difference between C1INH^{+/-} and C1INH^{-/-} mice is that, below a critical C1 inhibitor level, activation of the complement and contact systems is maximal, and a further decrease in the C1 inhibitor level has no additional effect. However, at least for the complement system, this is unlikely to be the case, because the C4 and hemolytic complement levels, although lower than in wild-type mice, are only moderately reduced. It also appears unlikely, therefore, that maximal activation of the contact system would occur. Another possibility is that the kallikrein substrate, high molecular weight kininogen, is depleted in both the C1INH^{+/-} and C1INH^{-/-} mice. The resolution of these questions awaits the development of appropriate reagents for these measurements.

The contributions of new therapeutic approaches toward definition of the pathophysiology of angioedema

Clinical trials using DX88 and Icatibant, the two new therapeutic agents described earlier, have provided further evidence supporting the hypothesis that angioedema in HAE is mediated by bradykinin. In the case of DX-88, two Phase II clinical trials have been completed, a third Phase II trial is nearly complete, and a Phase III trial is ongoing [108]. Icatibant has been used in one completed Phase II clinical trial, and two Phase III trials are

currently ongoing [109]. From the available data, both agents appear to be quite effective, in that they decrease the time to beginning of symptom relief. Furthermore, at appropriate doses, they both appear to be effective in nearly all patients. The available results from these trials, therefore, confirm the conclusions from the experiments in the C1 inhibitor knockout mice and indicate clearly that the primary mediator of attacks of angioedema in humans is bradykinin.

References

- [1] Landerman NS, Webster ME, Becker EL, et al. Hereditary angioneurotic edema. II. Deficiency of inhibitor for serum globulin permeability factor and/or plasma kallikrein. *J Allergy* 1962;33:330–41.
- [2] Donaldson VH, Evans RR. A biochemical abnormality in hereditary angioneurotic edema. *Am J Med* 1963;35:37–44.
- [3] Ratnoff O, Lepow I. Some properties of an esterase derived from preparations of the first component of complement. *J Exp Med* 1957;106:327–43.
- [4] Sim RB, Reboul A, Arlaud GJ, et al. Interaction of 125I-labelled complement components C1r and C1s with protease inhibitors in plasma. *FEBS Lett* 1979;97:111–5.
- [5] Ziccardi RJ. Activation of the early components of the classical complement pathway under physiological conditions. *J Immunol* 1981;126:1768–73.
- [6] de Agostini A, Lijnen HR, Pixley RA, et al. Inactivation of factor-XII active fragment in normal plasma: predominant role of C1-inhibitor. *J Clin Invest* 1984;93:1542–9.
- [7] Gallimore MJ, Amundsen E, Larsbraaten M, et al. Studies on plasma inhibitors of plasma kallikrein using chromogenic peptide substrate assays. *Thromb Res* 1979;16:695–703.
- [8] Gigli I, Mason JW, Colman RW, et al. Interaction of plasma kallikrein with the C1 inhibitor. *J Immunol* 1970;104:574–81.
- [9] Harpel PC, Lewin MF, Kaplan AP. Distribution of plasma kallikrein between C1 inactivator and a2-macroglobulin in plasma utilizing a new assay for a2-macroglobulin-kallikrein complexes. *J Biol Chem* 1985;260:4257–63.
- [10] Lewin MF, Kaplan AP, Harpel PC. Studies of C1 inactivator–plasma kallikrein complexes in purified systems and in plasma. Quantitation by an enzyme-linked differential antibody immunosorbent assay. *J Biol Chem* 1983;258:6415–21.
- [11] McConnell DJ. Inhibitors of kallikrein in human plasma. *J Clin Invest* 1972;51:1611–23.
- [12] Pixley RA, Schapira M, Colman RW. The regulation of human factor XIIa by plasma proteinase inhibitors. *J Biol Chem* 1985;260:1723–9.
- [13] Schapira M, Scott CF, Colman RW. Contribution of plasma protease inhibitors to the inactivation of kallikrein in plasma. *J Clin Invest* 1982;69:462–8.
- [14] van der Graaf F, Koedam JA, Bouma BN. Inactivation of kallikrein in human plasma. *J Clin Invest* 1983;71:149–58.
- [15] Harpel PC, Cooper NR. Studies on human plasma C1-inactivator-enzyme interactions. I. Mechanisms of interaction with C1s, plasmin and trypsin. *J Clin Invest* 1975;55:593–604.
- [16] Huisman LG, van Griensven JM, Kluft C. On the role of C1-inhibitor as inhibitor of tissue-type plasminogen activator in human plasma. *Thromb Haemost* 1995;73:466–71.
- [17] Ranby M, Bergstorf N, Nilsson T. Enzymatic properties of one and two chain forms of tissue plasminogen activator. *Thromb Res* 1982;27:175–84.
- [18] Ratnoff O, Pensky J, Ogston D, et al. The inhibition of plasmin, plasma kallikrein, plasma permeability factor, and the C1'r subcomponent of complement by serum C1' esterase inhibitor. *J Exp Med* 1969;129:315–31.

- [19] Sulikowski T, Patston PA. The inhibition of TNK-t-PA by C1-inhibitor. *Blood Coagul Fibrinolysis* 2001;12:75–7.
- [20] Aoki N, Moroi M, Matsuda M, et al. The behavior of alpha2-plasmin inhibitor in fibrinolytic states. *J Clin Invest* 1977;60:361–9.
- [21] Harpel PC. Alpha2-plasmin inhibitor and alpha2-macroglobulin-plasmin complexes in plasma. Quantitation by an enzyme-linked differential antibody immunosorbent assay. *J Clin Invest* 1981;68:46–55.
- [22] Cugno M, Cicardi M, Bottasso B, et al. Activation of the coagulation cascade in C1-inhibitor deficiencies. *Blood* 1997;89:3213–8.
- [23] Cugno M, Hack CE, Boer JPD, et al. Generation of plasmin during acute attacks of hereditary angioedema. *J Lab Clin Med* 1993;121:38–43.
- [24] Mast AE, Enghild JJ, Pizzo SV, et al. Analysis of the plasma elimination kinetics and conformational stabilities of native, proteinase-complexed, and reactive site cleaved serpins: comparison of alpha 1-proteinase inhibitor, alpha 1-antichymotrypsin, antithrombin III, alpha 2-antiplasmin, angiotensinogen, and ovalbumin. *Biochemistry* 1991;30:1723–30.
- [25] Bruch M, Weiss V, Engel J. Plasma serine proteinase inhibitors (serpins) exhibit major conformational changes and a large increase in conformational stability upon cleavage at their reactive sites. *J Biol Chem* 1988;263:16626–30.
- [26] Batra PP, Sasa K, Ueki T, et al. Circular dichroic study of conformational changes in ovalbumin induced by modification of sulhydryl groups and disulfide reduction. *J Protein Chem* 1989;8:609–17.
- [27] de Agostini A, Patston PA, Marottoli V, et al. A common neopeptide is created when the reactive center of C1-inhibitor is cleaved by plasma kallikrein, activated factor XII fragment, C1 esterase, or neutrophil elastase. *J Clin Invest* 1988;82:700–5.
- [28] Nuijens JH, Huijbregts CCM, Eerenberg-Belmer AJM, et al. Quantification of plasma factor XIIa–C1-inhibitor and kallikrein–C1-inhibitor complexes in sepsis. *Blood* 1988;72:1841–8.
- [29] Nuijens JH, Eerenberg-Belmer AJM, Huijbregts CCM, et al. Proteolytic inactivation of plasma C1 inhibitor in sepsis. *J Clin Invest* 1989;84:443–50.
- [30] Huntington JA, Read RJ, Carrell RW. Structure of a serpin-protease complex shows inhibition by deformation. *Nature* 2000;407:923–6.
- [31] Patston PA, Gettins P, Beechem J, et al. Mechanism of serpin action: evidence that C1 inhibitor functions as a suicide substrate. *Biochemistry* 1991;30:8876–82.
- [32] Bos IGA, Hack CE, Abrahams JP. Structural and functional aspects of C1-inhibitor. *Immunobiology* 2002;205:518–33.
- [33] Bock SC, Skriver K, Nielsen E, et al. Human C1 inhibitor: primary structure, cDNA cloning, and chromosomal localization. *Biochemistry* 1986;25:4292–301.
- [34] Coutinho M, Aulak KS, Davis AE III. Functional analysis of the serpin domain of C1 inhibitor. *J Immunol* 1994;153:3648–54.
- [35] Liu D, Gu X, Scafidi J, Davis AE III. N-linked glycosylation is required for C1 inhibitor-mediated protection from endotoxin shock in mice. *Infect Immun* 2004;72(4):1946–55.
- [36] Liu D, Cramer CC, Scafidi J, Davis AE III. N-linked glycosylation at Asn3 and the positively charged residues within the amino terminal domain of C1 inhibitor are required for its interaction with *Salmonella typhimurium* lipopolysaccharide and lipid A. *Infect Immun* 2005;73:4478–87.
- [37] Matsushita M, Endo Y, Fujita T. Cutting edge: complement-activating complex of ficolin and mannose-binding lectin-associated serine protease. *J Immunol* 2000;164:2281–4.
- [38] Matsushita M, Thiel S, Jensenius JC, et al. Proteolytic activities of two types of mannose-binding lectin associated serine protease. *J Immunol* 2000;165:2637–42.
- [39] Matsushita M, Endo Y, Hamasaki N, et al. Activation of the lectin complement pathway by ficolins. *Int Immunopharmacol* 2001;1:359–63.

- [40] Ambrus G, Gal P, Kojima M, et al. Natural substrates and inhibitors of mannan-binding lectin-associated serine protease—1 and —2: a study on recombinant catalytic fragments. *J Immunol* 2003;170:1374–82.
- [41] Jiang H, Wagner E, Zhang H, et al. Complement 1 inhibitor is a regulator of the alternative complement pathway. *J Exp Med* 2001;194:1609–16.
- [42] Colman RW, Schmaier AH. Contact system: a vascular biology modulator with anticoagulant, profibrinolytic, antiadhesive, and proinflammatory attributes. *Blood* 1997;90:3819–43.
- [43] Cochrane CG, Revak SD, Wuepper KD. Activation of Hageman factor in solid and fluid phases: a critical role of kallikrein. *J Exp Med* 1973;138:1564–83.
- [44] Griffin JH. The role of surface in the surface-dependent activation of Hageman factor (blood coagulation factor XII). *Proc Natl Acad Sci U S A* 1978;75:1998–2002.
- [45] Kaplan AP, Joseph K, Shibayama Y, et al. The intrinsic coagulation/kinin-forming cascade: assembly in plasma and cell surfaces in inflammation. *Adv Immunol* 1997;66:225–72.
- [46] Kirby E, McDevitt PJ. The binding of bovine factor XII to kaolin. *Blood* 1983;61:652–9.
- [47] Revak SD, Cochrane CG, Griffin JH. The binding and cleavage characteristics of human Hageman factor during contact activation: a comparison of normal plasma with plasma deficient in factor XI, prekallikrein or high molecular weight kininogen. *J Clin Invest* 1977;59:1167–75.
- [48] Mahdi F, Shariat-Madar Z, Todd RF III, et al. Expression and co-localization of cytokeratin 1 and urokinase plasminogen activator receptor on endothelial cells. *Blood* 2001;97:2342–50.
- [49] Joseph K, Ghebrehiwet B, Peerschke EIB, et al. Identification of the zinc-dependent endothelial cell binding protein for high molecular weight kininogen and factor XII: identity with the receptor that binds to the globular “heads” of C1q (gC1q-R). *Proc Natl Acad Sci U S A* 1996;93:8552–7.
- [50] Joseph K, Kaplan AP. Formation of bradykinin: a major contributor to the innate inflammatory response. *Adv Immunol* 2005;86:159–208.
- [51] Herwald H, Dedio J, Kellner R, et al. Isolation and characterization of the kininogen-binding protein p33 from endothelial cells. *J Biol Chem* 1996;271:13040–7.
- [52] Colman RW, Pixley RA, Najamunnisa S, et al. Binding of high molecular weight kininogen to human endothelial cells is mediated via a site within domains 2 and 3 of the urokinase receptor. *J Clin Invest* 1997;100:1481–7.
- [53] Hasan AAK, Zisman T, Schmaier AH. Identification of cytokeratin 1 as a binding protein and presentation receptor for kininogens on endothelial cells. *Proc Natl Acad Sci U S A* 1998;95:3615–20.
- [54] Joseph K, Tholanikunnel BG, Kaplan AP. Activation of the bradykinin-forming cascade on endothelial cells: a role for heat shock protein 90. *Int Immunopharmacol* 2002;2:1851–9.
- [55] Joseph K, Tholanikunnel BG, Kaplan AP. Heat shock protein 90 catalyzes activation of the prekallikrein-kininogen complex in the absence of factor XII. *Proc Natl Acad Sci U S A* 2002;99:896–900.
- [56] Shariat-Madar Z, Mahdi F, Schmaier AH. Identification and characterization of prolylcarboxypeptidase as an endothelial cell prekallikrein activator. *J Biol Chem* 2002;277:17962–9.
- [57] Shariat-Madar Z, Mahdi F, Schmaier AH. Assembly and activation of the plasma kallikrein/kinin system: a new interpretation. *Int Immunopharmacol* 2002;2:1841–9.
- [58] Schmaier AH. The plasma kallikrein-kinin system counterbalances the renin-angiotensin system. *J Clin Invest* 2002;109:1007–9.
- [59] Schmaier AH. The physiologic basis of assembly and activation of the plasma kallikrein/kinin system. *Thromb Haemost* 2004;91:1–3.
- [60] Kramer J, Rosen F, Colten H, et al. Transinhibition of C1 inhibitor synthesis in type I hereditary angioneurotic edema. *J Clin Invest* 1993;91:1258–62.
- [61] Ernst SC, Circolo A, Davis AE III, et al. Impaired production of both normal and mutant C1 inhibitor proteins in type I hereditary angioedema with a duplication in exon 8. *J Immunol* 1996;157:405–10.

- [62] Brickman CM, Frank MM, Kaliner M. Urine-histamine levels in patients with hereditary angioedema (HAE). *J Allergy Clin Immunol* 1988;82:402–6.
- [63] Colman RW. Activation of plasminogen by human plasma kallikrein. *Biochem Biophys Res Commun* 1969;35:273–9.
- [64] Goldsmith GH, Saito H, Ratnoff OD. The activation of plasminogen by Hageman factor (factor XII) and Hageman factor fragments. *J Clin Invest* 1978;62:54–60.
- [65] Revak SD, Cochrane CG, Bouma BN, et al. Surface and fluid-phase activities of two forms of activated Hageman factor produced during contact activation of plasma. *J Exp Med* 1978;147:719–29.
- [66] Kaplan AP, Austen KF. A pre-albumin activator of prekallikrein. *J Immunol* 1970;105:802–11.
- [67] Ghebrehiwet B, Silverberg M, Kaplan AP. Activation of the classical pathway of complement by Hageman factor fragment. *J Exp Med* 1981;153:655–76.
- [68] Donaldson DD. Mechanisms of activation of C1 esterase in hereditary angioneurotic edema plasma in vitro. The role of Hageman factor, a clot-promoting agent. *J Exp Med* 1968;127:411–29.
- [69] Heinz HP, Loos M. Activation of the first component of complement, C1: comparison of the effect of sixteen different enzymes on C1. *Immunobiology* 1983;165:175–85.
- [70] Francis CW, Brenner B, Leddy JP, et al. Angioedema during therapy with recombinant tissue plasminogen activator. *Br J Haematol* 1991;77:562–3.
- [71] Hoffmeister HM, Szabo S, Kastner C, et al. Thrombolytic therapy in acute myocardial infarction: comparison of procoagulant effects of streptokinase and alteplase regimens with focus on the kallikrein system and plasmin. *Circulation* 1998;98:2527–33.
- [72] Molinaro G, Gervais N, Adam A. Biochemical basis of angioedema associated with recombinant tissue plasminogen activator treatment: an in vitro experimental approach. *Stroke* 2002;33:1712–6.
- [73] Donaldson VH, Rosen FS. Action of complement in hereditary angioneurotic edema: the role of C1 esterase. *J Clin Invest* 1964;43:2204–13.
- [74] Carpenter CB, Ruddy S, Shehadeh IH, et al. Complement metabolism in man: hypercatabolism of the fourth (C4) and third (C3) components in patients with renal allograft rejection and hereditary angioedema (HAE). *J Clin Invest* 1969;48:1495–505.
- [75] Austen KF, Sheffer AL. Detection of hereditary angioneurotic edema by demonstration of a profound reduction in the second component of human complement. *N Engl J Med* 1965;272:649–56.
- [76] Quastel M, Harrison R, Cicardi M, et al. Behavior in vivo of normal and dysfunctional C1 inhibitor in normal subjects and patients with hereditary angioneurotic edema. *J Clin Invest* 1983;71:1041–6.
- [77] Lachmann P, Rosen F. The catabolism of C1-inhibitor and the pathogenesis of hereditary angioedema. *Acta Pathol Microbiol Immunol Scand* 1984;284:35–9.
- [78] Laurell AB, Lindegren J, Malmros I, et al. Enzymatic and immunochemical estimation of C1 esterase inhibitor in sera from patients with hereditary angioedema. *Scand J Clin Lab Invest* 1969;24:221–5.
- [79] Laurell AB, Martensson U, Sjöholm AG. C1 subcomponent complexes in normal and pathological sera studied by crossed immunoelectrophoresis. *Acta Pathol Microbiol Scand* 1976;84:455–64.
- [80] Donaldson VH, Ratnoff OD, Silva WDD, et al. Permeability-increasing activity in hereditary angioneurotic edema plasma. *J Clin Invest* 1969;48:642–53.
- [81] Donaldson VH, Merler E, Rosen FS, et al. A polypeptide kinin in hereditary angioneurotic edema plasma: role of complement in its formation. *J Lab Clin Med* 1970;76:986.
- [82] Donaldson VH. Kinin formation in hereditary angioneurotic edema (HANE) plasma. *Int Arch Allergy Appl Immunol* 1973;45:206–9.
- [83] Davies GE, Lowe JS. A permeability factor released from guinea pig serum by antigen-antibody precipitates. *Brit J Exp Pathol* 1960;41:335–44.

- [84] Klemperer MR, Donaldson VH, Rosen FS. Effect of C1 esterase on vascular permeability in man: studies in normal and complement-deficient individuals and in patients with hereditary angioneurotic edema. *J Clin Invest* 1968;47:604–11.
- [85] Ratnoff OD, Lepow IH. Complement as a mediator of inflammation. Enhancement of vascular permeability by purified C'1 esterase. *J Exp Med* 1963;118:681–98.
- [86] Davis AE III, Davis JS IV, Rabson AR, et al. Homozygous C3 deficiency: detection of C3 by radioimmunoassay. *Clin Immunol Immunopathol* 1977;8:543–50.
- [87] Strang CJ, Auerbach KS, Rosen FS. C1s-induced vascular permeability in C2-deficient guinea pigs. *J Immunol* 1986;137:631–5.
- [88] Strang C, Cholin S, Spragg J, et al. Angioedema induced by a peptide derived from complement component C2. *J Exp Med* 1988;168:1685–98.
- [89] Donaldson V, Rosen F, Bing D. Role of the second component of complement (C2) and plasmin in kinin release in hereditary angioneurotic edema (H.A.N.E.). *Trans Assoc Am Physicians* 1977;90:174–83.
- [90] Smith M, Kerr M. Cleavage of the second component of complement by plasma proteases: implications in hereditary C1-inhibitor deficiency. *Immunology* 1985;56:561–70.
- [91] Curd JG, Prograis LJ Jr, Cochrane CG. Detection of active kallikrein in induced blister fluids of hereditary angioedema patients. *J Exp Med* 1980;152:742–7.
- [92] Schapira M, Silver LD, Scott CF, et al. Prekallikrein activation and high-molecular-weight kininogen consumption in hereditary angioedema. *N Engl J Med* 1983;308:1050–3.
- [93] Curd JG, Yelvington M, Burridge N, et al. Generation of bradykinin during incubation of hereditary angioedema plasma [abstract]. *Mol Immunol* 1982;19:1365.
- [94] Fields T, Ghebrewhiet B, Kaplan A. Kinin formation in hereditary angioedema plasma: evidence against kinin derivation from C2 and in support of spontaneous formation of bradykinin. *J Allergy Clin Immunol* 1983;72:54–60.
- [95] Berrettini M, Lammle B, White T, et al. Detection of in vitro and in vivo cleavage of high molecular weight kininogen in human plasma by immunoblotting with monoclonal antibodies. *Blood* 1986;68:455–62.
- [96] Lammle B, Zuraw BL, Heeb MJ, et al. Detection and quantitation of cleaved and uncleaved high molecular weight kininogen in plasma by ligand blotting with radiolabeled plasma prekallikrein or factor XI. *Thromb Haemost* 1988;59:151–61.
- [97] Nielsen EW, Johansen HT, Hogasen K, et al. Activation of the complement, coagulation, fibrinolytic and kallikrein-kinin systems during attacks of hereditary angioedema. *Scand J Immunol* 1996;44:185–92.
- [98] Cugno M, Cicardi M, Coppola R, et al. Activation of factor XII and cleavage of high molecular weight kininogen during acute attacks in hereditary and acquired C1-inhibitor deficiencies. *Immunopharmacology* 1996;33:361–4.
- [99] Buhler R, Hovinga JK, Aebi-Huber I, et al. Improved detection of proteolytically cleaved high molecular weight kininogen by immunoblotting using an antiserum against its reduced 47 kDa light chain. *Blood Coagul Fibrinolysis* 1995;6:223–32.
- [100] Shoemaker LR, Schurman SJ, Donaldson VH, et al. Hereditary angioneurotic edema: characterization of plasma kinin and vascular permeability-enhancing activities. *Clin Exp Immunol* 1994;95:22–8.
- [101] Nussberger J, Cugno M, Amstutz C, et al. Plasma bradykinin in angio-oedema. *Lancet* 1998;351:1693–7.
- [102] Wisnieski JJ, Knauss TC, Yike I, et al. Unique C1 inhibitor dysfunction in a kindred without angioedema. I. A mutant C1 inhibitor that inhibits C1s but not C1r. *J Immunol* 1994;152:3199–209.
- [103] Zahedi R, Bissler JJ, Davis AE III, et al. Unique C1 inhibitor dysfunction in a kindred without angioedema. II. Identification of an Ala443-Val substitution and functional analysis of the recombinant mutant protein. *J Clin Invest* 1995;95:1299–305.
- [104] Zahedi R, Wisnieski J, Davis AE III. Role of the P2 residue of complement 1 inhibitor (Ala443) in determination of target protease specificity. *J Immunol* 1997;159:983–8.

- [105] Zahedi R, MacFarlane RC, Wisnieski JJ, Davis AE III. C1 inhibitor: analysis of the role of amino acid residues within the reactive center loop in target protease recognition. *J Immunol* 2001;167:1500–6.
- [106] Han ED, MacFarlane RC, Mulligan AN, et al. Increased vascular permeability in C1 inhibitor-deficient mice is mediated by the bradykinin type 2 receptor. *J Clin Invest* 2002;109:1057–63.
- [107] Agostini AD, Lijnen HR, Pixley RA, et al. Inactivation of factor XII active fragment in normal plasma. Predominant role of C1-inhibitor. *J Clin Invest* 1984;73:1542–9.
- [108] Dyax Corporation; 2004. Available at: www.dyax.com. Accessed July 6, 2006.
- [109] Jerini. Available at: www.jerini.com. Accessed July 6, 2006.