

Homozygous C1 inhibitor deficiency: The conclusion of a long search

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In 1888, Sir William Osler published a family tree of hereditary angioedema (HAE) in which individuals were afflicted in 5 consecutive generations.¹ The inheritance pattern was clearly that of an autosomal dominant disease. In 1963, Donaldson and Evans² noted that most patients with HAE have low levels of a plasma protein, the C1 inhibitor (C1-Inh).² The protein was known to inactivate C1, the first protein in the proinflammatory classical complement pathway. A few years later, Rosen et al³ established that low levels of the C1-Inh were characteristic of 85% of patients with HAE, whereas 15% had normal or increased levels of a dysfunctional C1-Inh. Further analysis indicated that plasma of all patients contained low levels of the normal protein. Thus patients with HAE have 1 normal and 1 mutated autosomal gene. For as yet incompletely understood reasons, the expected 50% level of the normal protein is not present. Until the report in this issue of the Journal, no patient has been described with a homozygous abnormality leading to almost a complete absence of plasma C1-Inh.⁴

The 17-kb gene for C1-Inh was cloned in 1989.⁵ It is present in a single copy on chromosome 11, and the gene contains 8 exons and 7 introns. Analyses of many families have led to the definition of a large number of substitutions, missense mutations, insertions, deletions, and duplications, particularly in exon 8 (see the hereditary angioedema database website at <http://hae.biomembrane.hu/index.php>).

The C1-Inh plasma protein is 478 amino acids long^{5,6} and contains 26% carbohydrate, making it one of the most heavily glycosylated proteins in plasma. It has 6 N-linked and 7 O-linked carbohydrate chains, of which the majority are present in the amino-terminal domain. Although the molecular weight of C1-Inh is calculated

to be 71 kd, it migrates as an approximately 105-kd protein on reduced gels, reflecting both the overall 2-domain shape of the protein and the abundant sugars.

This single-chain protein is a serpin, a serine protease inhibitor. One group of proteases have the amino acid serine within the active site, and this class of inhibitors is designed to inhibit these serine proteases. It has been termed a suicide inhibitor because it tightly complexes with the enzyme that it will destroy.^{6,7} The C1-Inh accomplishes this feat by presenting a bait sequence to the enzyme that resembles its natural substrates. The enzyme to be blocked thus cleaves the inhibitor at the bait sequence site, which is between an arginine at position 444 and a threonine at position 445. On cleavage, the C1-Inh undergoes a profound structural rearrangement. A highly reactive group becomes available that then forms a tight bond with the enzyme to be inhibited. In fact, by forming covalent bonds with each of the 2 C1r chains and each of the 2 C1s chains, the function of both the C1 and the C1-Inh is blocked (4 C1-Inh per C1). The C1-Inh–protease complex is then rapidly cleared by receptor-mediated binding and endocytosis (Fig 1).

The C1-Inh not only inhibits active C1 but also controls the activation of C1 (ie, the rate at which C1 acquires enzymatic activity).^{8,9} C1-Inh, like other members of this ancient gene family, regulates several other enzyme cascades in plasma.^{10,11} Although named for its ability to inactivate C1, it also inhibits mannan-associated serine proteases, the C1r/C1s equivalents of the mannan-binding lectin pathway.^{12,13} Furthermore, it controls the alternate complement pathway by binding at a nonprotease site on C3b.¹⁴ It also blocks Hageman factor, including its active fragments and the pathways activated by these fragments. These include kallikrein of the kinin-generating system, plasmin and tissue plasminogen activator of the fibrinolytic pathway, and Factor XI of the intrinsic clotting cascade.^{10,11} This wide-ranging antiprotease activity for mediator pathways of plasma has made it difficult to determine which pathway is responsible for the swelling in HAE.

Because no patient had ever been described with homozygous C1-Inh deficiency, it was assumed that such a deficiency would be lethal. However, Han et al¹⁵ generated a C1-Inh knockout mouse that developed normally. Homozygous deficient animals, though, were more sensitive to application of skin irritants, which caused vessel leakage that was not observed in normal animals. The heterozygous deficient animals were also more

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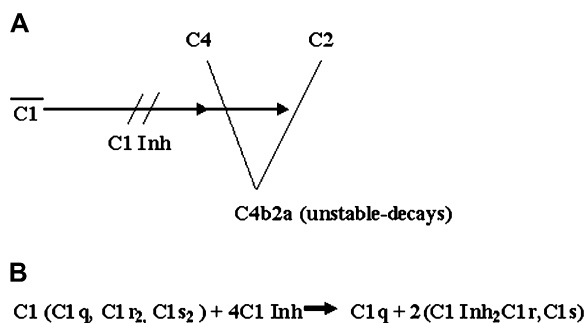


FIG 1. A, Action of C1 and C1-Inh in the complement cascade. Active C1 (designated by *overbar*) cleaves C4 and C2 to form a complex C4b2a, the classical and lectin pathway C3 convertase. This convertase is unstable and decays, releasing fragments that are removed from the circulation. Continuous activation of C1 in the absence of normal levels of C1-Inh depletes C4 and C2. **B,** Inactivation of C1 by the C1-Inh. Active C1 is inhibited by C1-Inh, which binds to C1 and dissociates the molecule, forming a free C1q and a covalent complex of C1-Inh C1r and C1s. The inhibitor-bound fragments are removed from the circulation.

sensitive to skin irritants. However, these deficient mice did not have episodic angioedema. The vessel leakage in response to skin irritants was corrected by infusion of human C1-Inh. If these animals were crossed to animals lacking the bradykinin 2 receptor or if given a kallikrein inhibitor (DX88), capillary leakage was prevented. These results strongly suggest that the kinin-generating pathway is responsible for the edema formation in HAE.¹⁶ This also fits with the clinical observation that angiotensin-converting enzyme inhibitors exacerbate this disease, presumably reflecting their role in the degradation of bradykinin.¹⁷

Although almost all patients with HAE possess 1 copy of a defective gene coding for an abnormal protein (they are heterozygotes), a single family was reported in which a promoter-region defect in the C1-Inh gene in both the father and the mother led to low levels of C1-Inh in the afflicted child.¹⁸ This is the only patient who has had an abnormality of both genes, but he did not have an abnormality in the coding sequence.

In this issue of the Journal, 2 individuals in one family with a homozygous C1-Inh gene mutation featuring a defective protein are reported.⁴ The defect does not lead to a failure to synthesize or secrete C1-Inh, as is true of most gene defects in HAE, nor does it lead to the formation of totally nonfunctional protein, as is the case with many other mutations. The mutation instead appears to lead to the synthesis of a hypomorphic or poorly functioning protein. Consequently, this family is not an example of a true null situation in which there is absolutely no protein produced.

The family tree of the proband demonstrates consanguinity and multiple heterozygous relatives and a second "deficient" individual. The 2 homozygous individuals have so little circulating C1-Inh that they appear to have type 1 HAE. The 2 heterozygotes appear to have type 2

HAE because they have normal levels of a C1-Inh protein that functions poorly.

The clinical and biochemical findings in this family are surprising. Clinically, one of the homozygous deficient patients has few and the other has had no angioedema attacks. The heterozygotes are free of symptoms. The reasons for this are by no means clear. The authors have considered the possibility, suggested by the work of Zahedi et al,¹⁹ that the protein synthesized inhibits kallikrein but is defective in inhibiting C1. They suggest that this is not the case, but this point requires further study.

The biochemical abnormalities observed in these patients are also not what one might have expected. It has been a truism that patients with HAE have normal C1 and C1q levels but low levels of C4 and C2.²⁰ The 2 homozygous deficient individuals in this study have very low levels of C4, and many, but not all, of the heterozygous patients have low levels of C4 as well.⁴ C2 was not tested. The unexpected and striking finding, though, is that the 2 individuals with abnormalities of both C1-Inh genes and who express small quantities of the hypomorphic C1-Inh have low or undetectable C1q levels. As noted above, the C1-Inh, on binding to C1r and C1s, takes apart the 3-subunit C1 molecule, leaving C1q behind. The C1r/C1s–C1-Inh complexes are rapidly removed from the circulation. In the absence of a normal amount of the C1-Inh, as is true of most cases of HAE, this does not efficiently occur, and activated C1 continues to circulate.

Low levels of C1q with abnormalities of C1-Inh had only been previously observed in patients with acquired C1-Inh deficiency syndrome.^{21,22} In most of these patients, an mAb is produced that binds the C1-Inh near the site at which the covalent bond forms with the enzyme to be inhibited. In most of these patients, there is a large amount of C1-Inh in the circulation that is cleaved at the active substrate site but that has not formed a covalent bond. These patients have a prominent, circulating, 76-kd, inactive C1-Inh fragment. The patients in this family also have such a fragment.⁴

Why C1q levels are low in patients with acquired C1-Inh deficiency is not known. It does not seem to be as simple as the autoantibody C1-Inh complex binding C1q, but that is a possibility. Perhaps the antibody binds to the C1-Inh–C1 complex and then the C1q, and other products are all prematurely cleared from the circulation. How can individuals with a reduced level of a hypomorphic protein have such a low C1q level in their circulation? Perhaps the hypomorphic form binds to the activated C1 but does not have a sufficient binding affinity to dissociate the molecule. Under such a circumstance, the entire complex might be removed from the circulation.

The findings in this important article will cause us to rethink our understanding of the biochemistry of the disease and the activity of the C1-Inh.⁴ In studies of this sort, the gold standard is to transfect the abnormal gene into cells, express the protein, and isolate it to study function *in vitro*. In theory, the gene could be introduced into C1-Inh-deficient mice; the biochemical effect of the abnormality should also be considered. All these studies

await future investigation. The authors are to be congratulated for their careful evaluation of this family and for the first identification of a patient homozygous for abnormalities in the C1-Inh protein.

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