

From <sup>a</sup>the Division of Rheumatology, Allergy and Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC; <sup>b</sup>the University of North Carolina Hospitals, Chapel Hill, NC; <sup>c</sup>Bristol-Myers Squibb, Plainsboro, NJ; <sup>d</sup>the Division of Oncology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC; and <sup>e</sup>the Asthma and Allergic Disease Center, University of Virginia Medical School, Charlottesville, Va. E-mail: tarra002@med.unc.edu.

Disclosure of potential conflict of interest: B. Mirakhor is employed by Bristol-Myers Squibb. T. A. E. Platts-Mills has served as a consultant for Dyson Vacuum Cleaners and Indoor Biotechnologies and has received research support from ImClone. T. K. Tarrant has received research support from the National Institute of Allergy and Infectious Diseases/National Institutes of Health and the American College of Rheumatology. The rest of the authors have declared that they have no conflict of interest.

## REFERENCES

- Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;351:337-45.
- Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous cell carcinoma of the head and neck. *N Engl J Med* 2006;354:567-78.
- Patel DD, Goldberg RM. Cetuximab-associated infusion reactions: pathology and management. *Oncology* 2006;20:1373-82.
- Erbitux (cetuximab) [drug product label]. New York: ImClone Systems Incorporated and Bristol-Myers Squibb; 2007.
- O'Neil BH, Allen R, Spiegel DR, Stinchcombe TE, Moore DT, Berlin JD, et al. High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina: association with atopic history. *J Clin Oncol* 2007;25:3644-8.
- Chung CH, Mirakhor B, Chan E, Le Q, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose- $\alpha$ -1,3-galactose. *N Engl J Med* 2008;358:1109-17.
- Cancer Therapy Evaluation Program. Common terminology criteria for adverse events, v3.0 (CTCAE). Bethesda: National Cancer Institute; 2006.
- Nielsen DL, Pfeiffer P, Jensen BV. Re-treatment with cetuximab in patients with severe hypersensitivity reactions to cetuximab. Two case reports. *Acta Oncol* 2006;45:1137-8.
- Cheifetz A, Mayer L. Monoclonal antibodies, immunogenicity, and associated infusion reactions. *Mt Sinai J Med* 2005;72:250-6.

Available online November 21, 2008.  
doi:10.1016/j.jaci.2008.09.046

## Angioedema and estrogen-dependent angioedema with activation of the contact system

### To the Editor:

Angioedema attacks occurring in hereditary or acquired deficiency of the C1 esterase inhibitor (C1-INH) present together with uncontrolled activation of the contact system.<sup>1</sup> The contact system consists of Factor XII, prekallikrein, and high-

molecular-weight kininogen (HMWK). Activation of the contact system generates activated Factor XII (Factor XIIa), which transforms prekallikrein into kallikrein, which in turn cleaves bradykinin from HMWK. Bradykinin causes vasodilatation through binding to bradykinin B2 receptors. Bradykinin is short-lived and is essentially inactivated by kininases I and II. C1-INH deficiency favors unchecked protease activity of Factor XIIa and kallikrein, leading to HMWK cleavage.

Recently, a novel type of angioedema has been described in women.<sup>2,3</sup> Clinical symptoms resembled those seen in C1-INH deficiency, but C1-INH was found to be normal in concentration and function. Two forms were tentatively characterized.<sup>4</sup> The first type is called *estrogen-dependent angioedema* and has a clear familial component, with symptoms occurring only during pregnancy, oral contraceptive use, or estrogen replacement therapy. The second type, also affecting women, is tentatively named *estrogen-associated angioedema* and also has a strong familial component, but several of the patients with this type experienced angioedema attacks unrelated to oral contraceptive use or pregnancy. A mutation in exon 9 of the gene coding for Hageman factor (XII) was described recently in several German families and 1 French family with estrogen-dependent angioedema.<sup>5,6</sup> This mutation was shown to be associated with an important gain in function of Factor XII.<sup>6</sup>

We studied 4 female patients who had been found to have a decrease in residual kallikrein activity during angioedema attacks. Citrated plasma and serum samples had been frozen during and between angioedema episodes. All 4 women were taking estrogen-containing oral contraceptives at the time of the angioedema attacks. Patients 1 and 4 had repeated angioedema attacks over a 30-year period. Both experienced angioedema of the pharynx or localized angioedema of a limb. Some episodes were related to local trauma or upper respiratory tract infections. Patient 1 also had angioedema during a pregnancy and 2 episodes of ileus and ascites. A maternal aunt of patient 1 suffocated because of upper respiratory tract obstruction at the age of 20 years. Patients 2 and 3 had angioedema episodes over a 2-year period. Their angioedema episodes improved after surgical treatment of dental root inflammation.

Serum levels of C1q were defined by radial immunodiffusion, and C4, C3, and C1-INH levels were determined by means of

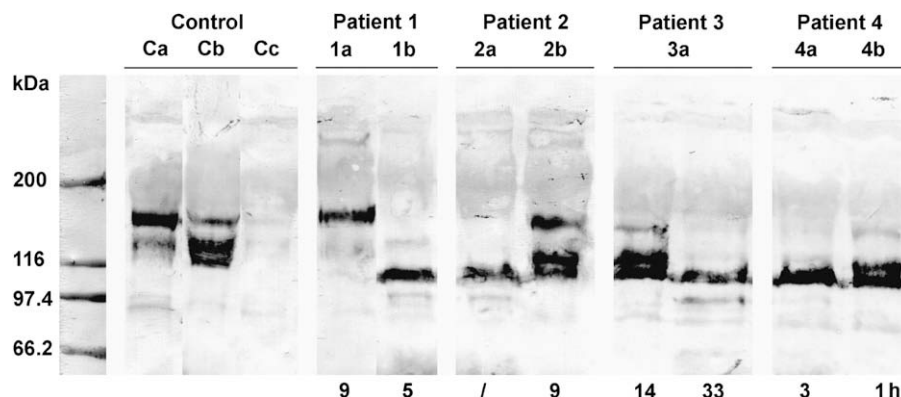


**FIG 1.** HMWK immunoblot using an anti-heavy chain mAb with normal human plasma Ca (1 strong band at about 130 kD), glass-activated plasma Cb (additional bands at 115 kD and 100 kD), and HMWK-deficient plasma Cc (no protein band is detected) and immunoblots with patient plasma samples taken during angioedema episodes of patient 1 (1a and 1b), patient 2 (2a and 2b), patient 3 (3a), and patient 4 (4a and 4b). h, Time in hours after start of edema. Except for episode 1a, all the episodes show total (1b, 2a, 3a, 4a, and 4b) or partial (2b) cleavage of HMWK.

**TABLE I.** Complement and contact system values of patients 1, 2, 3, and 4 during angioedema episodes

| Episode                        | Patient 1 |      | Patient 2 |     | Patient 3 |      | Patient 4 |      | Normal values |
|--------------------------------|-----------|------|-----------|-----|-----------|------|-----------|------|---------------|
|                                | 1a        | 1b   | 2a        | 2b  | 3a        |      | 4a        | 4b   |               |
| Time after start of angioedema | 9 h       | 5 h  | PA        | 9 h | 14 h      | 33 h | 3 h       | 1 h  |               |
| C1q                            | ND        | 98   | ND        | ND  | 127       | 111  | 100       | ND   | 5-25 mg/dL    |
| C4                             | 19        | 22   | 15        | 15  | 20        | 16   | 37        | 45   | 15-30 mg/dL   |
| C3                             | 78        | 78   | 119       | 95  | 103       | 126  | 91        | 133  | 80-140 mg/dL  |
| C1-INH antigen                 | 12        | 14   | 28        | 19  | 19        | 22   | 14        | 18   | 15-24 mg/dL   |
| C1-INH F                       | 50%       | 53%  | 100%      | 32% | 45%       | 69%  | 74%       | 95%  | 70% to 130%   |
| Kallikrein F                   | 65%       | 106% | 100%      | 30% | 67%       | 111% | 104%      | 104% | 80% to 120%   |

*h*, Time in hours after the beginning of angioedema; *PA*, protracted angioedema; *ND*, not done; *F*, activity.

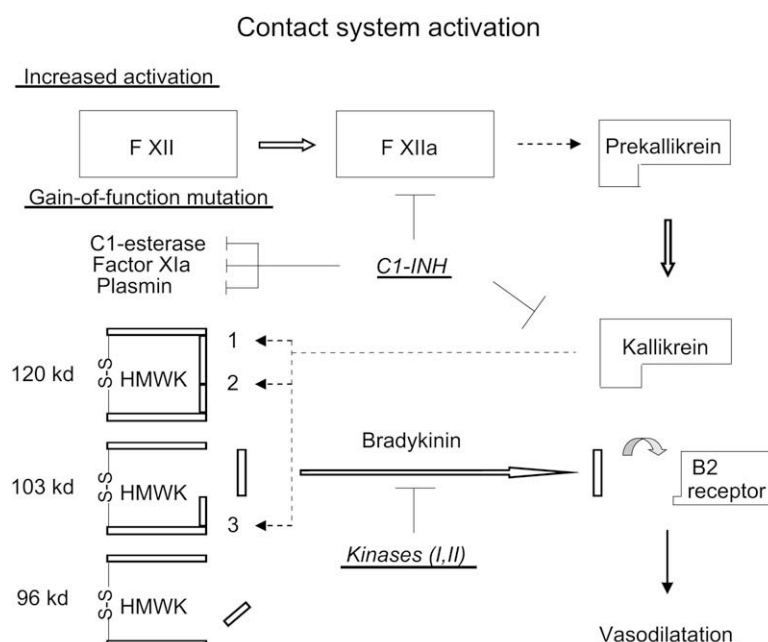


**FIG 2.** Diagram showing contact system components and their main interactions. Activation of Factor XII (*F XII*) generates Factor XIIa (*F XIIa*), which transforms prekallikrein into kallikrein, which in turn cleaves bradykinin from HMWK. HMWK cleavage by kallikrein at sites 1 and 2 generates bradykinin and a 103-kd moiety, and further cleavage at site 3 results in an HMWK moiety of 96 kd. Interaction of bradykinin with the B2 receptor leads to vasodilatation. —→, activation or cleavage; ⊥, inactivation or degradation. C1-INH inactivates Factor XIIa and kallikrein. Kininases I and II inactivate bradykinin.

nephelometry with specific antisera. After activating prekallikrein, kallikrein activity was determined by using an amidolytic assay with the substrate benzoyl-prolyl-phenylalanyl-arginine-4-nitrilide (Chromozym P.K.; Roche Applied Science, Basel, Switzerland). C1-INH activity was determined by measuring its capacity to inhibit the transformation of a chromogenic substrate by C1-esterase (Berichrom C1-Inhibitor; Siemens Medical Solutions, Erlangen, Germany). Kallikrein activity and C1-INH activity are given as the percentage of standard plasma levels. HMWK cleavage was analyzed by immunoblotting human plasma with a previously described mouse mAb directed at the heavy chain of HMWK.<sup>7</sup> The pattern of reactivity of this mAb with normal plasma, contact-activated plasma, and kininogen-deficient plasma (Fitzgerald trait; Sigma, St Louis, Mi) is shown in Fig 1 under "Control." Intact glycosylated HMWK with a molecular weight of 120 kd (here with an apparent molecular weight of 130 kd) is first cleaved by kallikrein to a 103-kd molecule (here 115 kd)

with liberation of bradykinin and then cleaved to a moiety of 96 kd (here apparent 100 kd).<sup>8</sup> Exon 9 of Factor XII was amplified and sequenced to test for the mutation at position 1032 in exon 9. Oligonucleotide primers were designed on the basis of the DNA reference sequence D0119. Primers F12for (5'GAACCCG GACAACGACATCCGCC) and F12rev (5'ACAGACTCTTGCG GAGCCGCTG) amplified a 407-base-pair fragment of exons 9 and 10. Complement levels, as well as C1-INH and kallikrein activity values, are shown in Table I. Results of HMWK immunoblotting are shown in Fig 1. In all patients HMWK is completely or almost completely cleaved during angioedema attacks. This suggests that contact system activation with HMWK cleavage and bradykinin liberation leads to angioedema attacks. In patient 1 sequencing of exon 9 showed the presence of a C 1032 to A mutation responsible for a threonine to lysine change in position 309 of Factor XII. This mutation has been described in estrogen-dependent angioedema and found to be associated with a gain-of-function

mutation of Hageman factor.<sup>5,6</sup> Patients 2, 3, and 4 did not have this mutation.

The 4 patients had normal C1-INH values at baseline, and values of the early complement components C1q, C4, and C3 were normal during the angioedema episodes. These findings are strong evidence against congenital or acquired C1-INH deficiency. During several episodes of angioedema, the patients had evidence of contact system activation, which was characterized by a decrease in kallikrein function and cleavage of HMWK. The attacks were commonly accompanied by decreased C1-INH function, as measured by a decreased capacity to inhibit the cleavage of a chromogenic substrate by C1-esterase. C1-INH levels remained normal when tested by means of nephelometry.

Because the patients showed no sign of C1-INH deficiency in the normal state, it can be assumed that contact system activation is the primary pathogenic event in their angioedema attacks (Fig 2). It is of interest to note that patient 1 was found to have the threonine to lysine mutation at position 309 of Factor XII described with estrogen-dependent angioedema. This mutation was correlated with a considerable increase in activity of Factor XII. These data document that contact system activation occurred during angioedema attacks in patient 1 with estrogen-dependent angioedema. The other 3 patients did not have this mutation in Factor XII; however, angioedema attacks happened while they were taking estrogen-containing oral contraceptives. The worsening effect of estrogens on hereditary angioedema has been described by several authors.<sup>2-4</sup> In women taking oral contraception, there is an increase in Factor XII, prekallikrein, and HMWK values and a decrease in C1-INH levels.<sup>9</sup> Because these known estrogen-related effects are insufficient to explain contact system activation and angioedema attacks in these women, other genetic or nongenetic factors that played a triggering role remain to be investigated.

*François Hentges, MD<sup>a,b</sup>*  
*Christiane Hilger, PhD<sup>b</sup>*  
*Marianne Kohlen, BSc<sup>a</sup>*  
*Georges Gilson, PhD<sup>c</sup>*

From <sup>a</sup>the Unit of Immunology and Allergology, Centre Hospitalier de Luxembourg, <sup>b</sup>the Laboratory of Immunogenetics and Allergology, CRP-Santé, and <sup>c</sup>the Laboratory of Biochemistry and Immunopathology Centre Hospitalier de Luxembourg, Luxembourg. E-mail: [hentges.francois@chl.lu](mailto:hentges.francois@chl.lu).

Supported by grant 88-03 of the Ministry of Research Luxembourg.

Disclosure of potential conflict of interest: F. Hentges receives grant support from CRP-Santé Luxembourg, Fonds National de la Recherche, and the Ministry of Research. G. Gilson received grant support from CRP-Santé Luxembourg. The rest of the authors have declared that they have no conflict of interest.

## REFERENCES

1. Hack E. Pathogenesis of angioedema attacks. In: Farkas H, editor. Hereditary and acquired angioedema: problems and progress: proceedings of the Third C1 Esterase Inhibitor Deficiency Workshop and Beyond. *J Allergy Clin Immunol* 2004; 114(suppl):S75-9.
2. Binkley KE, Davis AE III. Clinical, biochemical and genetic characterization of a novel estrogen-dependent inherited form of angioedema. *J Allergy Clin Immunol* 2000;106:546-50.
3. Bork K, Barnstedt SE, Koch P, Traupe H. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet* 2000;356:213-7.
4. Binkley K, Davis AE III. Estrogen-dependent and estrogen-associated inherited angioedema (previously HAE type III). In: Farkas H, editor. Hereditary and acquired angioedema: problems and progress: proceedings of the Third C1 Esterase Inhibitor Deficiency Workshop and Beyond. *J Allergy Clin Immunol* 2004; 114(suppl):S62-4.
5. Dewald G, Bork K. Missense mutation in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. *Biochem Biophys Res Commun* 2006;343:1286-9.
6. Cichon S, Martin L, Hennies HC, Müller F, Van Driessche K, Karpushova A, et al. Increased activity of coagulation Factor XII (Hageman Factor) causes hereditary angioedema type III. *Am J Hum Genet* 2006;79:1098-104.
7. Hentges F, Kohlen M, Grigioni F, Reichert P, Humbel R, Schneider F. Production and characterisation of monoclonal antibodies directed against high and low molecular weight kininogens. *Bull Soc Sci Med Grand Duche Luxemb* 1994; 1321:9-17.
8. Berrettini M, Lämmle B, White T, Heeb MJ, Schwarz HP, Zuraw B, 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.
9. Hoem NO, Johannesen S, Hauge G, Rud AC, Sandem S, Briseid K. Contact activation factors in plasma from women using oral contraceptives- increased levels of factor XII, kinin- free high molecular weight kininogen and acetone-activated kallikrein. *Thromb Res* 1991;64:427-34.

doi:10.1016/j.jaci.2008.10.056