

Activation of the contact system and fibrinolysis in autoimmune acquired angioedema: A rationale for prophylactic use of tranexamic acid

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C1-inhibitor deficiency results in bouts of mucocutaneous edema and may be inherited (hereditary angioedema) or acquired (acquired angioedema [AAE]). The two forms have the same clinical picture but differ in the response to treatment. Prophylaxis with antifibrinolytic agents produces better results in the acquired form than in the inherited form, in which androgen derivatives are more effective. It is hypothesized that activation of the contact and fibrinolytic systems is involved in the pathogenesis of attacks. We evaluated these two systems in plasma from eight patients with AAE and anti-C1-inhibitor autoantibodies (autoimmune AAE) by measuring the cleavage of high molecular weight kininogen and the complexes formed by plasmin and its inhibitor α_2 -antiplasmin. We also measured complement parameters, autoantibody titer, and cleaved C1-inhibitor (relative molecular mass = 96,000), because autoantibodies to C1-inhibitor are known to facilitate its cleavage by proteases. Plasma was obtained from patients in remission, during prophylactic treatment with the antifibrinolytic agent tranexamic acid (2 to 4.5 gm/day) and also from two patients during acute attacks of edema. Levels of cleaved high molecular weight kininogen and antiplasmin-plasmin complexes in patients with AAE were both higher in basal conditions, during treatment, and during acute attacks than those in normal subjects ($p < 0.001$). The cleaved inactive form of C1-inhibitor was also present in all patients in all three conditions. Therapy with antifibrinolytic agents reduced the frequency and intensity of symptoms without significantly changing any of the biochemical parameters. Our data demonstrate that in patients with autoimmune AAE there is continuous activation of contact and fibrinolytic systems, which might be counteracted by antifibrinolytic agents, as indicated by the favorable effects of prophylactic treatment with tranexamic acid. (J ALLERGY CLIN IMMUNOL 1994;93:870-6.)

Key words: Acquired angioedema, autoimmunity, C1-inhibitor, high molecular weight kininogen, plasmin, tranexamic acid

The deficiency of C1-inhibitor can be inherited (hereditary angioedema [HAE])¹ or acquired (acquired angioedema [AAE]), which typically affects individuals in adulthood.^{2, 3} The latter condition has been mainly reported in association with lymphoproliferative disorders⁴ and as an autoimmune form with autoantibodies against C1-

Abbreviations used

AAE:	Acquired angioedema
AMCA:	Trans-4-aminomethylcyclohexane-1-carboxylic acid
APP:	Antiplasmin-plasmin
HAE:	Hereditary angioedema
HK:	High molecular weight kininogen
M _r :	Relative molecular mass
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

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inhibitor.⁵⁻⁷ The massive consumption of C1 and low plasma levels of C1q, which is normal in HAE,^{8, 9} are characteristics of AAE. In the presence of a lymphoproliferative disease, it appears that interaction between the idiotype of monoclo-

TABLE 1. Plasma levels of C1-inhibitor, C4, C1q, cleaved HK and APP complexes in eight patients with AAE during remission

Patient No.	C1-inhibitor function (% NHPP)	C1-inhibitor antigen (% NHPP)	C4 antigen (% NHPP)	C1q antigen (% NHPP)	Cleaved HK (% of total HK)	APP complexes (ng/ml)
1	<5	19	<5	<5	82	784
2	<5	58	<5	<5	66	932
3	<5	23	<5	<5	80	542
4	22	30	<5	93	52	552
5	<5	27	<5	<5	67	453
6	20	29	<5	<5	97	370
7	<5	19	<5	<5	69	386
8	<5	25	<5	<5	86	1351
NR	67-133	72-128	56-145	62-138	11-33	172-302

NHPP, Normal human pooled plasma; NR, normal range.

nal immunoglobulins and antiidiotypic antibodies causes increased consumption of C1q and C1-inhibitor.¹⁰ In autoimmune AAE the autoantibodies facilitate the cleavage of C1-inhibitor by its target protease(s), preventing the formation of a stable protease-inhibitor complex. This interaction eventually results in high plasma levels of a catabolic product of C1-inhibitor with a relative molecular mass (M_r) of 96,000.^{11, 12}

C1-inhibitor regulates the classical complement pathway, the contact system, and fibrinolysis.¹³ In hereditary C1-inhibitor deficiency various degrees of activation of these three systems and their roles in the pathogenesis of symptoms have been reported.¹⁴⁻¹⁹ We recently demonstrated that attacks of edema in HAE are associated with the cleavage of high molecular weight kininogen (HK) and with plasmin generation.¹⁹ These data supported the previous *in vitro* observation that the fibrinolytic system is involved in the release of the vasoactive mediators responsible for the symptoms.²⁰ In acquired C1-inhibitor deficiency the pathogenesis of angioedema is supposed to be the same as in HAE. However, we have no data at present that describe the consequences of acquired C1-inhibitor deficiency to the contact and fibrinolytic systems.

Symptoms in HAE are prevented by attenuated androgens that act by increasing C1-inhibitor synthesis.^{21, 22} We recently reported that these drugs have no effect in the majority of patients with autoimmune AAE in whom the administration of the antifibrinolytic agent tranexamic acid (trans-4-aminomethylcyclohexane-1-carboxylic acid [AMCA]) produces results.²³ This drug, an analog more potent than ϵ -amino-caproic acid, blocks the lysine-binding sites of plasminogen

and plasmin, impairing their fibrinolytic activity,²⁴ and it has been introduced for the treatment of HAE as a plasmin antagonist but with limited success.^{25, 26}

In this study we investigated the contact, fibrinolytic, and complement systems in patients with autoimmune AAE in basal conditions, during attacks, and during prophylactic treatment with AMCA. The results demonstrate that in these patients the contact and fibrinolytic systems are already activated in basal conditions, which is a possible explanation for the effectiveness of antifibrinolytic agents in this disorder.

METHODS

We studied eight patients (two women and six men; age range, 55 to 73 years) with autoimmune AAE. Clinical data have been previously reported.²³ Samples were obtained from all patients in a period of remission without any treatment, during long-term prophylaxis with 2 to 4.5 gm tranexamic acid per day (six patients) and, in two patients, during acute attacks of edema involving the face and the neck. Ten healthy subjects (seven men and three women; age range, 48 to 69 years) served as a control group.

Blood was drawn by clean venipuncture with minimal stasis and collected in siliconized Vacutainer tubes (Becton Dickinson, Plymouth, U.K.) to which ethylenediaminetetraacetic acid (10 mmol/L) and hexadimethrine bromide (0.05% wt/vol) had been added. Tubes were centrifuged at 1300g at room temperature for 10 minutes, and plasma aliquots were stored in polystyrene tubes at -70°C until tests were performed.

Autoantibodies to C1-inhibitor were assessed by an ELISA method.⁶ Briefly, plasma dilutions were incubated in microtiter plates coated with C1-inhibitor protein (Immuno AG, Vienna, Austria). After repeated washing, autoantibodies were detected by class-specific monoclonal antibody anti-human immunoglobulin

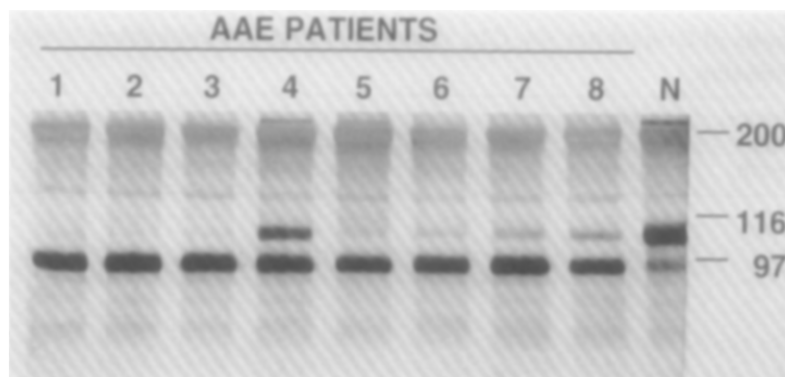


FIG. 1. Immunoblotting of C1-inhibitor in plasma from a normal subject (*N*) and from eight patients with AAE. Normal subject (*N*) has a major band with M_r of 106,000, corresponding to native C1-inhibitor and a faint band with M_r of 96,000, corresponding to cleaved C1-inhibitor. The patients (identified by number in Table I) have a major band at M_r of 96,000, corresponding to cleaved C1-inhibitor, whereas the native band (M_r = 106,000) is faint or absent.

TABLE II. Plasma levels of APP complexes, cleaved HK, C1-inhibitor function and antigen, C4 antigen, and anti-C1-inhibitor antibody titers in healthy subjects and patients with AAE during remission without any treatment (basal AAE), during treatment with tranexamic acid (AMCA AAE), or during acute attacks (AA AAE)

	Normal subjects (<i>n</i> = 10)	Basal AAE (<i>n</i> = 8)	AMCA AAE (<i>n</i> = 6)	AA AAE (<i>n</i> = 2)
APP complexes (ng/ml)	269 (172-302)	547* (370-1351)	562* (321-614)	617-1444
Cleaved HK (% of total HK)†	27 (11-33)	75* (52-97)	78* (44-82)	89-90
C1-inhibitor function (% of normal)	98 (67-133)	<5* (<5-22)	<5* (<5-11)	<5
C1-inhibitor antigen (% of normal)	102 (72-128)	26* (19-58)	31* (19-39)	25-29
C4 antigen (% of normal)	104 (56-145)	<5*	<5*	<5
Anti-C1-inhibitor auto-antibody (titer)‡	—	1200 (320-6400)	850 (118-4000)	550-4250

Values are expressed as median with range in parentheses. In the two patients with AAE during attacks (AA AAE) single values are reported.

*Statistical significance versus normal subjects ($p < 0.001$).

†Cleaved HK (% of total HK) in 10 patients with HAE during remission was 24% (10% to 48%) and in five patients with HAE during acute attacks 75% (40% to 90%) ($p = 0.003$).

‡Reciprocal of plasma dilution producing 50% of maximal binding.

(Sigma Chemical Co., St. Louis, Mo.). C1-inhibitor activity was measured by a chromogenic assay (Immuno AG). C1-inhibitor, C4, and C1q antigens were assessed by radial immunodiffusion (Nor-Partigen, Behringwerke, Marburg, Germany).

Cleaved HK has been evaluated with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting analysis. Plasma was subjected to nonreducing SDS-PAGE (8% wt/vol). After electrophoretic transfer of proteins from gel onto a polyvinylidene difluoride membrane (Immobilon; Millipore, Bedford, Mass.), HK forms were identified with goat polyclonal anti-light-chain-HK (Nordic, Tilburg, The Netherlands) and visualized with a biotin-

ylated donkey anti-goat antibody (Gibco BRL, Gaithersburg, Md.). The apparent molecular mass of proteins was estimated by comparison with high molecular weight protein markers from Bio-Rad Laboratories (Richmond, Calif.). HK bands were quantified by densitometry (Cliniscan 2; Helena Laboratories, Milan, Italy).

Immunoblotting analysis of C1-inhibitor was carried out under the same conditions as those described for HK, but goat polyclonal anti-C1-inhibitor was used as first antibody (Atlantic Antibodies, Scarborough, Maine). Antiplasmin-plasmin (APP) complexes were measured in plasma with an ELISA method (Behringwerke AG, Marburg, Germany).

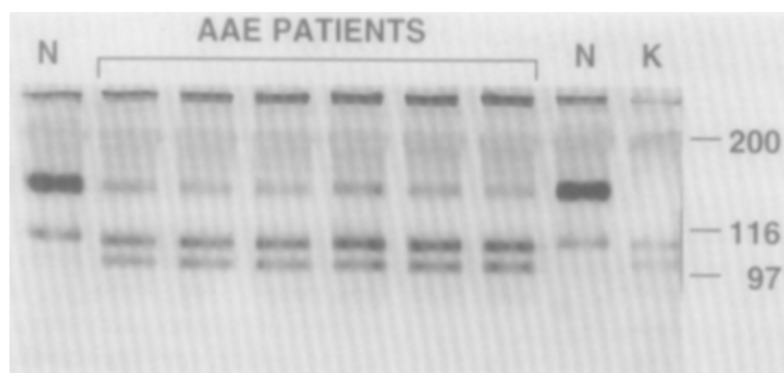


FIG. 2. Immunoblotting of HK in plasma from normal subjects (*N*) and from patients with AAE. The normal pattern shows a major band with M_r of 130,000 and a faint band with M_r of 107,000. In patients there is reduction of the band with M_r of 130,000, increase of the band with M_r of 107,000, and appearance of a band with M_r of 98,000. This pattern, which corresponds to that of cleaved HK, can be obtained by activating human normal plasma with kaolin (*K*) in vitro.

Statistical analysis

The significance of differences between groups was determined by the nonparametric test of Wilcoxon-Mann-Whitney. Correlations were assessed by the Spearman rank analysis. A *p* value of less than 0.05 was considered to indicate a statistically significant difference or correlation.

RESULTS

Individual data for APP complexes, cleaved HK, C1-inhibitor function and antigen, and C1q antigen, C4 antigen in eight patients with autoimmune AAE are reported in Table I.

In all patients antigenic C1-inhibitor levels were variably higher than the functional levels. Immunoblotting analysis of C1-inhibitor demonstrated that the cleaved form with an M_r of 96,000 was by far the main band in the plasmas of all patients (Fig. 1). C4 and C1q plasma levels were below 5% of normal except in one patient who had normal levels of C1q. Anti-C1-inhibitor autoantibodies were present in all eight patients (2 IgA, 2 IgM, 4 IgG), with plasma titers (expressed as the reciprocal of the dilution producing 50% of maximal binding) from 320 to 6400 (Table II). In basal conditions all patients had higher values of APP complexes than normal subjects ($p < 0.001$) (Table II).

AMCA treatment (2 to 4.5 gm/day), which was given to seven patients, reduced the frequency and intensity of symptoms in six, as already reported,²³ but did not change C1-inhibitor, C4, C1q, or autoantibody titers. APP complexes and cleaved HK, measured in the six patients who benefited from the treatment, also remained unchanged (Table II). No correlation was evident in

patients with AAE between HK and APP, and HK and APP were not correlated with C1-inhibitor, C4, or autoantibody titer.

SDS-PAGE analysis of HK (Fig. 2) in normal pooled plasma produced a major band with an M_r of 130,000 and a faint one with an M_r of 107,000, corresponding to the native HK and its catabolic product. The incubation of normal pooled plasma with kaolin (an activator of the contact system) cleaves HK and results in a pattern in which the band with an M_r of 130,000 disappears, the band with an M_r of 107,000 increases, and a band with an M_r of 98,000 appears (Fig. 2, lane 9). The cleavage of HK in our samples was quantified by densitometry, considering the absorbance of the two bands with M_r s of 107,000 and 98,000, representing cleaved HK, as percentage of total HK (sum of the three bands with M_r s of 130,000, 107,000, and 98,000). Levels of cleaved HK were significantly greater in patients with autoimmune AAE than in normal subjects ($p < 0.001$), but there were no significant differences among patients under different conditions (basal, AMCA treatment, attack) (Table II).

DISCUSSION

The functional defect of C1-inhibitor, the only physiologic inhibitor of C1, results in activation of the classical complement pathway. It has been reported that such activation is markedly greater in patients with the acquired C1-inhibitor defect than in those with the inherited one.^{8, 27} However, no explanation of this difference has been provided.

In this study we investigated contact phase and

fibrinolysis, the other two systems regulated by C1-inhibitor, in eight patients with autoimmune AAE. To measure contact activation, we used an immunoblotting analysis of HK, which was slightly modified from the original description of Berrettini et al.²⁸ With similar techniques, evidence of contact activation has been reported during attacks in HAE and to a lesser extent in various inflammatory conditions.^{19, 28-30} The activation of fibrinolysis was studied by measuring the plasma levels of APP complexes, a sensitive and specific method reflecting the *in vivo* generation of plasmin.^{19, 31-33} With these two methods, we have now demonstrated that there is activation of contact and fibrinolytic systems in basal conditions in patients with autoimmune AAE. This finding was unexpected, because a deficiency of C1-inhibitor does not lead *per se* to activation of contact phase or fibrinolysis as we previously showed in patients with HAE¹⁹ (even if a small activation in HAE cannot be excluded). However, it indicates that in AAE all three systems controlled by C1-inhibitor (complement, contact phase, and fibrinolysis) are more unstable than in HAE, displaying signs of activation.

Our present data do not provide any easy explanation for the difference. A relationship between C1 consumption and contact system activation is suggested by the observation that the patient (no. 4, Table I) with normal C1q levels is the one with lowest percentage of HK cleavage. This patient also has relatively higher C1-inhibitor function levels. However, low C1-inhibitor function levels cannot account for the activation of C1 and HK cleavage detected in AAE. In fact, there is neither C1 depletion nor activation of contact phase in patients with HAE in basal conditions, even when functional C1-inhibitor is undetectable.¹⁹ Therefore in addition to C1-inhibitor deficiency, some other mechanism for C1 depletion and contact activation must be acting in patients with AAE. This mechanism, which is still unknown, probably does not act through the immune complex formed by C1-inhibitor and autoantibody, because Cochrane et al.³⁴ demonstrated that immune complexes do not activate the contact system; however, their methods could not rule out the possibility of a weak activation.

The increased levels of APP complexes in our patients are not surprising, because generation of plasmin has been demonstrated after contact activation *in vivo* and *in vitro*.^{35, 36}

The attacks in AAE and HAE are clinically identical. Although the mediator of symptoms is

still controversial,^{20, 37} it has been shown in HAE that it has kinin-like characteristics³⁸ and that it is released on the activation of the complement, contact, and fibrinolytic systems.^{14, 16, 20} Our present data for the two patients with AAE during an attack show that both HK cleavage and APP complexes were elevated but without any clear-cut difference between the levels during attacks and basal conditions. However, the small number of patients and the preexistent activation of the contact and fibrinolytic systems probably hampered the demonstration of the attack-related generation of plasmin and HK cleavage as we demonstrated in patients with HAE.¹⁹

In a recent article²³ we described the high effectiveness of the antifibrinolytic agent AMCA for prophylaxis of attacks in patients with AAE. Such effectiveness is clearly greater than that reported for patients with HAE.³ The pharmacologic effects of AMCA are obtained by blocking the lysine-binding sites on the molecules of plasminogen and plasmin, which prevents their binding to fibrin, resulting in the inhibition of fibrinolysis.²⁴ However, the binding of AMCA to the circulating plasminogen molecule induces structural changes,³⁹ which paradoxically facilitate the formation of plasmin in the fluid phase. The AMCA-plasmin complex is rapidly inactivated by $\alpha 2$ -antiplasmin and $\alpha 2$ -macroglobulin.³⁹ The mechanism of action of AMCA in C1-inhibitor deficiencies probably depends on its antiplasmin activity, which impairs the release of vasoactive mediators. Patients with AAE already have signs of activation of fibrinolysis in basal conditions, and this can facilitate attacks, making large amounts of plasmin available in the fluid phase. The counteraction of active plasmin by AMCA may provide a clue to understanding its particular effectiveness in these patients. However, we did not detect any changes in the APP complexes during long-term treatment with AMCA at doses proven to be antifibrinolytic.²⁴ The facilitation of the activation of plasminogen by AMCA and the generation of an AMCA-plasmin complex, which is rapidly bound to $\alpha 2$ -antiplasmin,³⁹ may reconcile the apparent contradiction detected in patients with AAE, in whom a supposed antiplasmin effect coexists with elevated APP plasma levels. On the other hand, the possibility that an antifibrinolytic agent acts to prevent C1 activation in patients with AAE has been considered. Such an effect, demonstrated *in vitro* a long time ago,⁴⁰ does not seem to be relevant in our patients, because AMCA treatment does not induce any

increase in C1q levels. Moreover, the persistence during AMCA treatment of cleaved C1-inhibitor, derived from the interaction of C1-inhibitor bound to the autoantibody with active C1,^{6, 11} further supports the view that C1 is not influenced by antifibrinolytic prophylaxis. It has been recently demonstrated that AMCA is active as a result of kringle domain interaction on plasmin molecule.⁴¹ Because the kringle domain is sheared by various serin proteases, an unknown activity of AMCA on proteases other than plasmin could not be excluded.

REFERENCES

- Donaldson VH, Evans RR. A biochemical abnormality in hereditary angioneurotic edema: absence of serum inhibitor of C1-esterase. *Am J Med* 1963;31:36-44.
- Caldwell JR, Ruddy S, Schur PH, Austen KJ. Acquired C1 inhibitor deficiency in lymphosarcoma. *Clin Immunol Immunopathol* 1972;1:39-52.
- Agostoni A, Cicardi M. Hereditary and acquired C1-inhibitor deficiency: biological and clinical characteristics in 235 patients. *Medicine* 1992;71:206-15.
- Gelfand JA, Boss GR, Conley CL, Reinhart R, Frank MM. Acquired C1 esterase inhibitor deficiency and angioedema: a review. *Medicine* 1979;58:311-8.
- Jackson J, Sim RB, Whelan A, Feighery C. An IgG autoantibody which inactivates C1 inhibitor. *Nature* 1986;313:722-4.
- Alsenz J, Bork K, Loos M. Autoantibody mediated acquired deficiency of C1 inhibitor. *N Engl J Med* 1987;316:1360-6.
- Frank MM, Malbran A, Simms H, et al. Acquired angioedema type II: a new autoimmune disease [Abstract]. *Clin Res* 1987;35:641A.
- Melamed J, Alper CA, Cicardi M, Rosen FS. The metabolism of C1 inhibitor and C1q in patients with acquired C1 inhibitor deficiency. *J ALLERGY CLIN IMMUNOL* 1986;77:322-6.
- Donaldson VH, Evans RR. Action of complement in hereditary angioneurotic edema: the role of C1 esterase. *J Clin Invest* 1964;43:2204-13.
- Geha RS, Quinti I, Austen KF, Cicardi M, Sheffer A, Rosen FS. Acquired C1 inhibitor deficiency associated with antiidiotypic antibody to monoclonal immunoglobulins. *N Engl J Med* 1985;312:534-40.
- Jackson J, Sim RB, Whaley K, Feighery C. Autoantibody facilitated cleavage of C1 inhibitor in autoimmune angioedema. *J Clin Invest* 1989;83:698-707.
- Alsenz J, Lambris JD, Bork K, Loos M. Acquired C1 inhibitor (C1 INH) deficiency type II. *J Clin Invest* 1989;83:1794-9.
- Davis AE III. C1 inhibitor and hereditary angioneurotic edema. *Ann Rev Immunol* 1988;6:595-628.
- Shapira 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.
- Zuraw BL, Curd CG. Demonstration of modified inactive first component of complement (C1) inhibitor in the plasma of C1-inhibitor deficient patients. *J Clin Invest* 1986;78:567-75.
- 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.
- Cugno M, Bergamaschini L, Uziel L, et al. Hemostasis contact system and fibrinolysis in hereditary angioedema (C1-inhibitor deficiency). *J Clin Chem Clin Biochem* 1988;6:423-7.
- Kaufman N, Page JD, Pixley RA, Schein R, Schmaier AH, Colman RW. α 2-Macroglobulin-kallikrein complexes detect contact system activation in hereditary angioedema and human sepsis. *Blood* 1991;70:1053-62.
- Cugno M, Hack CE, de Boer JP, Eerenberg AJM, Agostoni A, Cicardi M. Generation of plasmin during acute attacks of hereditary angioedema. *J Lab Clin Med* 1993;121:38-43.
- Donaldson VH, Rosen FS, Bing DH. Role of the second component of complement (C2) and plasmin in kinin release in hereditary angioneurotic edema (HANE) plasma. *Trans Assoc Am Physicians* 1977;40:174-83.
- Gelfand JA, Sherins RJ, Alling DW, Frank MM. Treatment of hereditary angioedema with danazol. Reversal of clinical and biochemical abnormalities. *N Engl J Med* 1976;295:1444-8.
- Falus A, Fehér KG, Walcz E, et al. Hormonal regulation of complement biosynthesis in human cell lines. I. Androgens and gamma-interferon stimulate the biosynthesis and gene expression of C1-inhibitor in human cell lines U 937 and Hep G2. *Mol Immunol* 1990;27:191-5.
- Cicardi M, Bisiani G, Cugno M, Späth P, Agostoni A. Autoimmune C1 inhibitor deficiency: report of eight patients. *Am J Med* 1993;95:169-75.
- Verstrate M. Clinical application of inhibitors of fibrinolysis. *Drugs* 1985;29:236-61.
- Sheffer AL, Austen KF, Rosen FS. Tranexamic acid therapy in hereditary angioneurotic edema. *N Engl J Med* 1972;287:452-4.
- Agostoni A, Marasini B, Cicardi M, Martignoni GC, Uziel L, Pietrogrande M. Hepatic function and fibrinolysis in patients with hereditary angioedema undergoing long-term treatment with tranexamic acid. *Allergy* 1978;33:211-6.
- Oltvai ZN, Wong ECC, Atkinson JP, Tung KSK. C1 inhibitor deficiency: molecular and immunologic basis of hereditary and acquired angioedema. *Lab Invest* 1991;65:381-8.
- Berrettini M, Lämmle B, White T, et al. Detection of in vivo cleavage of high molecular weight kininogen in human plasma by immunoblotting with monoclonal antibodies. *Blood* 1986;68:455-62.
- Chhibber G, Cohen A, Lane S, Farber A, Meloni FJ, Schmaier AH. Immunoblotting of plasma in a pregnant patient with hereditary angioedema. *J Lab Clin Med* 1990;115:112-21.
- Lämmle B, Zuraw BL, Heeb MJ, et al. Detection and quantitation of cleaved and uncleaved high molecular weight kininogen in plasma by ligand blotting with radio-labeled plasma prekallikrein or factor XI. *Thromb Haemost* 1988;59:151-61.
- Harpel PC. α 2-Plasmin inhibitor and α 2-macroglobulin-plasmin complexes in plasma. Quantitation by an enzyme-linked differential antibody immunosorbent assay. *J Clin Invest* 1981;68:46-55.
- Holvoet P, de Boer A, Verstreken M, Collen D. An enzyme-linked immunosorbent assay (ELISA) for the measurement of plasmin- α 2-antiplasmin complex in hu-

- man plasma: application to the detection of in vivo activation of the fibrinolytic system. *Thromb Haemost* 1986; 56:124-7.
33. Levi M, de Boer JP, Roem D, ten Cate JW, Hack CE. Plasminogen activation in vivo upon intravenous infusion of DDAVP. Quantitative assessment of plasmin- α 2-anti-plasmin complex with a novel monoclonal antibody based radioimmunoassay. *Thromb Haemost* 1992;67:111-6.
34. Cochrane CG, Wuepper KD, Aiken BS, Revak SD, Spiegelberg HL. The interaction of Hageman factor and immune complexes. *J Clin Invest* 1972;51:2736-45.
35. Kluft C, Dooijeward G, Emeis JJ. Role of the contact system in fibrinolysis. *Semin Thromb Hemost* 1987;13:50-68.
36. Kaplan AP, Silverberg M. The coagulation-kinin pathway of human plasma. *Blood* 1987;70:1-15.
37. Fields T, Ghebrehiwet B, Kaplan AP. 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.
38. Donaldson VH, Ratnoff OD, Dia da Silva W, Rosen FS. Permeability-increasing activity in hereditary angio-neurotic edema plasma. *J Clin Invest* 1969;48:642-53.
39. Astedt B. Clinical pharmacology of tranexamic acid. *Scand J Gastroenterol* 1987;22(suppl 137):22-5.
40. Soter NA, Austen KF, Gigli I. Inhibition by ϵ -aminocaproic acid of the activation of the first component of the complement system. *J Immunol* 1975;114:928-32.
41. Anonick PK, Vasudevan J, Gonias SL. Antifibrinolytic activities of α -N-acetyl-L-lisin methyl ester, ϵ -aminocaproic acid, and tranexamic acid. Importance of kringle interaction and active site inhibition. *Arterioscler Thromb* 1992; 12:708-16.

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