
Case report

Polyclonal autoantibodies against C1 inhibitor in a case of acquired angioedema

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Background: Angioedema attributable to acquired C1 inhibitor (C1-INH) deficiency is a rare disease related to lymphoproliferative disorders or autoantibodies to C1 inhibitor. We describe a patient with angioedema and autoantibodies to C1 inhibitor.

Objective: To study the characteristics of autoantibodies to C1-INH in a patient with acquired angioedema.

Methods: Autoantibodies to C1-INH were measured by enzyme-linked immunoadsorbent assay. Immunoglobulin (Ig)G autoantibody was purified by affinity chromatography on a protein G agarose column. We developed an enzyme-linked immunoadsorbent assay to determine whether the autoantibodies were directed against the C1-INH active center.

Results: IgM and mainly C1-INH IgG autoantibodies were detected; both had κ and λ chains. No monoclonal component was detected. The autoantibodies were directed against the C1-INH active center. After various treatment strategies were attempted, an effective clinical response was attained with antifibrinolytic therapy.

Conclusion: A case of acquired angioedema because of C1-INH deficiency was found to be attributable to the presence of polyclonal autoantibodies to C1-INH.

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INTRODUCTION

C1 inhibitor (C1-INH) is a plasma glycoprotein, with a molecular weight of 105 kD and a serum concentration of approximately 0.2 g/L. It belongs to the superfamily of serine protease inhibitors (serpins) and inactivates proteases of the contact phase (factor XIIa), coagulation factor XIa, complement (activated C1r and C1s), tissue plasminogen activator, and kallikrein. Therefore, C1-INH is an important inhibitor of the inflammatory response because uncontrolled activation of the

aforementioned systems caused by C1-INH deficiency induces liberation of vasoactive molecules that result in mucosal and dermal edema.^{1,2}

Angioedema attributable to acquired C1-INH deficiency, a condition in which serum C1-INH levels are in the range noted for patients with hereditary deficiency of this protein, was first described in 1972 as a clinical entity in association with lymphoproliferative disorders.³ In 1986, autoimmune C1-INH deficiency was described,⁴ in which the patient's serum contained an autoantibody to C1-INH, but this finding was not associated with lymphoproliferation. This condition was labeled as type II acquired angioedema, and the variant associated with lymphoproliferative diseases was called type I. The autoantibodies are oligoclonal or monoclonal, and the syndrome is characterized by low serum levels of C1q,

C4, and C2; normal serum levels of C3; and normal or reduced serum levels of C1-INH protein, which is functionally inactive. Cicardi et al⁵ suggested that the distinction between type I and type II acquired angioedema may be artificial, inasmuch as some patients may have paraproteinemia or lymphoreticular neoplasms several years after the onset of angioedema.

The most important clinical symptoms are edema and obstruction of upper respiratory airways. Recurrent episodes of subcutaneous or mucosal edema (or both) usually manifest during the fourth decade of life. Laboratory findings are characterized by low levels of C4, C1-INH, and C1q.⁶

In this report, we describe a 71-year-old male patient who had recurrent edema, in whom laboratory studies showed a complement profile compatible with the diagnosis of type II acquired angioedema: C3, 73.2 mg/dL; C4, <1.67 mg/dL; C1-INH, 4.8 mg/dL; and C1q, 60 μ g/mL. The functional activity of C1-INH was undetectable. The presence of anti-C1-INH autoantibodies was also evidenced by indirect enzyme-linked immunoadsorbent assay (ELISA).

CASE REPORT

A 71-year-old man underwent evaluation in our allergy outpatient clinic because of recurrent severe episodes of cutaneous angioedema once a month for 1 year. The edema was localized to the arms, legs, and genitalia and had evolved to blisters in some sites. Because of the severity of the edema, the patient had difficulty walking and

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leading a normal life. Classic angioedema treatment with corticosteroids and antihistamines had yielded no response. At the time of initial examination, no edema of the glottis was present. The cutaneous edema had been associated with abdominal episodes, consisting of colicky pain, nausea, and vomiting. There was no evidence of neoplasms or hereditary angioedema, and the patient had not taken angiotensin-converting enzyme inhibitors. Arterial hypertension was treated with chlorthalidone. A surgical procedure had been necessary for peritonitis and appendicitis 30 years earlier.

Skin prick tests were negative to a battery of inhalants, foods, and *Anisakis simplex*. Similarly, patch tests were negative to the standard True Test series.

Laboratory studies of the blood showed normal findings on the complete blood cell count, erythrocyte sedimentation rate, and biochemical analyses. Serum protein electrophoresis revealed a slightly decreased γ -globulin fraction, with no monoclonal component. Serum immunoglobulins (Ig) were as follows: IgG, 478 mg/dL (normal, 520 to 1,740); IgA, 89 mg/dL (normal, 77 to 362); IgM, 194 mg/dL (normal, 40 to 260); and IgE, 470 kU/L (normal, 80 to 120). Serum complement studies showed the following results: C3, 73.2 mg/dL (normal, 70 to 200); C4, <1.67 mg/dL (normal, 14 to 47); C1-INH, <4.8 mg/dL (normal, 14 to 40); C1q, 60 μ g/mL (normal, 118 to 244); and CH₅₀, <50 U/mL (normal, 250 to 400). Serum immune complexes, antinuclear antibodies, and rheumatoid factor were negative. On serum immunoelectrophoresis, no monoclonal gammopathy was detected.

Serologic studies were negative for syphilis, human immunodeficiency virus, hepatitis A and C, *Echinococcus*, and Epstein-Barr virus, and positive for hepatitis B (antibody to hepatitis B surface antigen positive, hepatitis B surface antigen negative, antibody to hepatitis B core antigen positive), parvovirus (IgG), herpes simplex (IgG), and cytomegalovirus (IgG). Three different stool samples were negative for parasites. X-ray

studies of the thorax and paranasal sinuses showed normal findings.

Autoantibodies to C1-INH were positive for IgG and IgM; there were no IgE or IgA antibodies to C1-INH. The patient was diagnosed with angioedema attributable to acquired C1-INH deficiency as a result of C1-INH autoantibodies.

On screening studies for malignant lesions, thoracic and abdominal computed tomography showed normal results. No evidence was found of a lymphoproliferative malignant process. The initial long-term prophylaxis was with stanozolol (2 mg every 8 hours), but 3 months later, the patient had facial edema in conjunction with edema of the glottis, which ceased after intravenous treatment with 500 IU of C1-INH. Thus, the stanozolol regimen was discontinued, and treatment with tranexamic acid was initiated (500 mg every 8 hours). Clinically, the patient improved, and during the subsequent 2 years, the frequency of the episodes of angioedema decreased to one in 3 months and the severity diminished.

During an acute attack, a biopsy of the skin blisters was performed to exclude pemphigus and bullous pemphigoid. The histologic finding was a subepidermal blister with negative results on direct immunofluorescence microscopy. Autoantibodies to interepidermal substance and to epidermal basement membrane were also negative.

The only offspring of the patient, a daughter, underwent studies for complement levels. The results of these studies were normal.

MATERIAL AND METHODS

Blood from the patient was obtained by venipuncture under standard conditions. To preserve C1-INH functional activity, samples were collected with a mixture of protease inhibitors (ethylenediaminetetraacetic acid, benzamidine, and soybean trypsin inhibitor). Plasma was obtained by centrifugation of the blood in polypropylene tubes, and aliquots were immediately stored at -70°C until used. When the samples were thawed, they were handled

in polypropylene tubes to minimize contact system activation.

Serum Ig levels were measured by nephelometry (Array, Beckman Instruments, Fullerton, CA). Serum total IgE was measured by microparticle enzyme immunoassay (IMx, Abbott Diagnostics, Abbott Park, IL). Protein electrophoresis was performed by capillary electrophoresis (Paragon CZE 2000, Beckman).

For complement determinations, C3 and C4 were measured by nephelometry, and C1-INH was determined by radial immunodiffusion (Dade Behring, Marburg, Germany). C1-INH function was measured as described by de Smet et al,⁷ C1q by radial immunodiffusion (The Binding Site, Birmingham, UK), and CH₅₀ by a hemolytic assay.⁸

Lymphocyte subsets were analyzed by fluorescence flow cytometry on a FACSort Cytometer with use of Cell Quest Software (Becton Dickinson, Franklin Lakes, NJ). Monoclonal antibodies anti-CD3, anti-CD4, anti-CD8, and anti-CD19, as well as anti- κ and anti- λ , which were also from Becton Dickinson.

Detection of Autoantibodies Directed Against C1-INH

ELISA plate wells (Costar, Bucks, UK) were coated overnight at 4°C with purified human C1-INH (Berinert P, Centeon Pharma GmbH, Marburg, Germany) by adding 100 μ L C1-INH (10 μ g/mL) in coating buffer (0.05 mol/L carbonate-bicarbonate buffer, pH 9.6). After washing in isotonic phosphate-buffered saline (PBS; pH 7.4) containing 0.1% vol/vol Tween 20 (PBS-T), unreacted sites were blocked by the addition of 150 μ L of PBS containing 3% gelatin (BDH, Poole, England) wt/vol. After 1 hour at room temperature, the wells were washed three times with PBS-T, and serum samples (100 μ L) were added at several dilutions in PBS-bovine serum albumin (BSA) 0.1% (from 1:50 to 1:12,800). After several washings, two types of antibodies at the appropriate dilution were added: 1) peroxidase coupled with goat anti-human-IgG, an-

ti-IgM, or anti-IgA; or 2) biotin coupled with goat IgG anti-human IgE (BioSource, Camarillo, CA). For revealing the anti-IgE, an additional step was included containing streptavidin-alkaline phosphatase (Sigma Chemical, St. Louis, MO). Plates were incubated for 1 hour at room temperature as they were shaken. Each enzyme coupled to the antibodies was developed with specific substrate, and the optical density was determined. Further, the integrity of the active center of C1-INH was evaluated by testing the ability to bind C1s; at each assay, a control was introduced containing biotin-labeled C1s (Calbiochem, San Diego, CA), followed by the addition of streptavidin-poly-horseradish peroxidase (CLB Biotechnology, Amsterdam, the Netherlands) and substrate.

IgG Antibody Purification

IgG from the patient's serum was purified by affinity chromatography on a protein G agarose column (Genzyme, Cambridge, MA), according to the manufacturer's instructions. The purity of the preparation was tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the concentration of IgG was calculated by measuring absorbance at 280 nm and assuming an extinction coefficient of 13.6. To test the possible monoclonal origin of anti-C1-INH antibodies, sera and the IgG fraction containing C1-INH autoantibodies were retitrated with use of rabbit antibodies against each light chain (anti- κ or anti- λ chains; Operon, Zaragoza, Spain), followed by the addition of peroxidase goat anti-rabbit Igs (BioSource). Further, purified IgG was subjected to isoelectric focusing, in accordance with the method of Keir et al,⁹ with a mixture of ampholines of two ranges from 3 to 10 and 8 to 10.5 followed by transference to a nitrocellulose membrane. The membrane was incubated with an anti-human IgG labeled with alkaline phosphatase and revealed with the appropriate substrate. This IgG was used in the following experiments.

Inhibition of C1-INH autoantibody binding to immobilized C1-INH in-

duced by soluble C1-INH. An aliquot (0.025 mg/mL) of purified IgG in PBS-BSA 0.1% was incubated for 30 minutes at 37° C with variable amounts (1 to 50 nmol) of soluble C1-INH (Berinert P) or with the same

amounts of human serum albumin. Subsequently, 100 μ L of each mixture was added to C1-INH-coated wells and incubated at 37° C for 30 minutes. Then the presence of IgG bound to the plates was detected with a peroxidase goat anti-

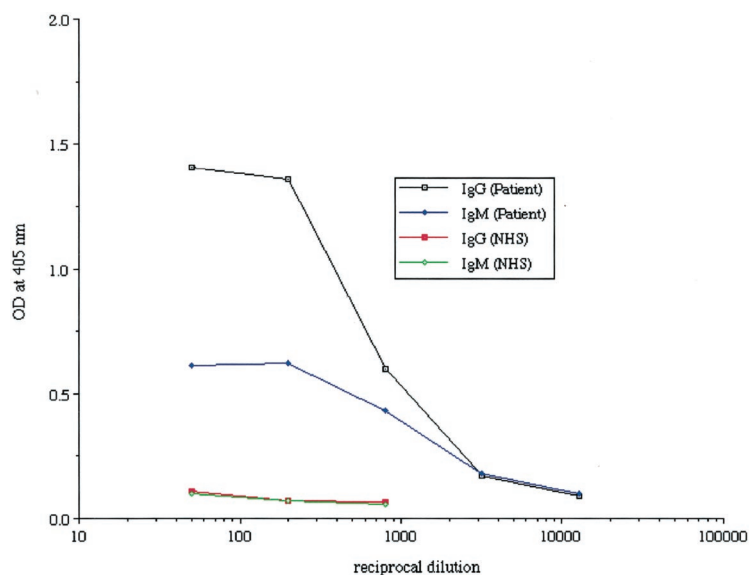


Figure 1. Assessment for autoantibodies against C1-INH by ELISA in patient with acquired angioedema and in normal human serum (NHS). Plastic plates were coated overnight with 1 μ g C1-INH, and then 3% gelatin was added for saturating the wells. Serum samples were added at several dilutions, from 1:50 to 1:12,800. The antibodies were developed with either peroxidase goat anti-human IgG or peroxidase goat anti-human IgM. The peroxidase was developed with the appropriate substrate. One representative experiment of five is plotted. OD, optical density.

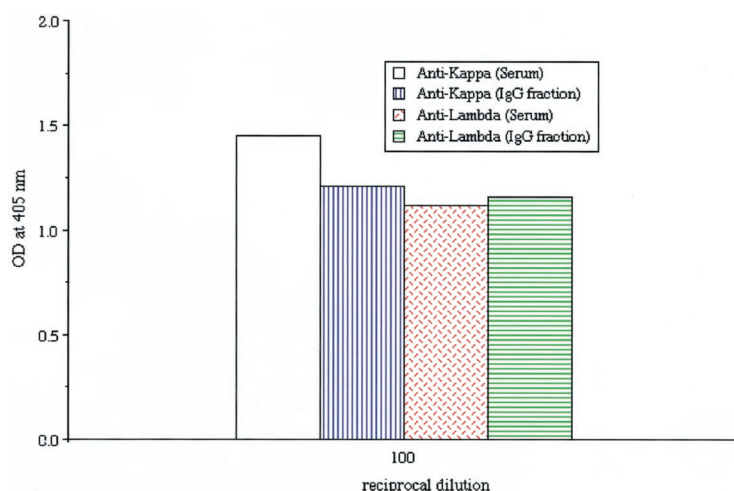


Figure 2. Reactivity of the patient's serum and IgG fraction with anti- κ and anti- λ chain antibodies, as evaluated by ELISA. Plastic plates were coated overnight with 1 μ g of C1-INH, as described in legend for Figure 1. Then serum or IgG fraction, 1:100 diluted, was incubated with C1-INH-coated plates. Subsequently, a rabbit anti- κ or anti- λ polyclonal antiserum was added. After washing, a peroxidase goat anti-rabbit Igs was added and developed with 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) as substrate. OD = optical density.

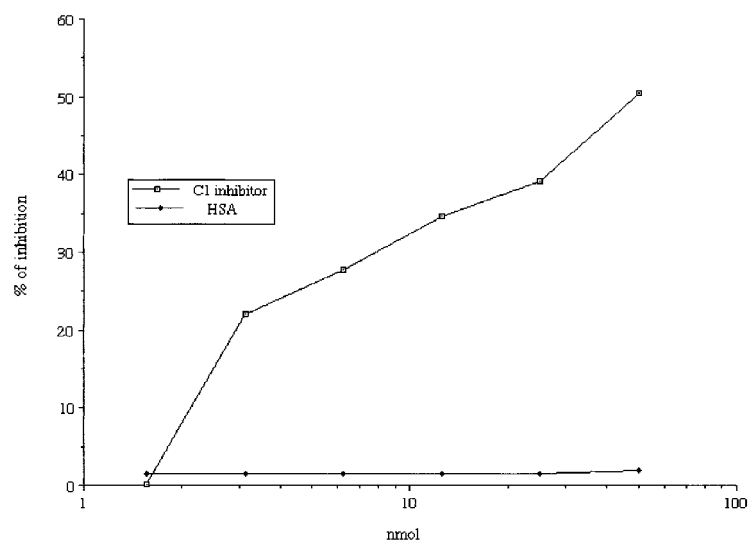


Figure 3. Dose-dependent competition of purified IgG with soluble C1-INH. An aliquot (100 μ L) of purified IgG (0.025 mg/mL) in PBS (pH 7.2) was added to different amounts of C1-INH (1 to 50 nmol) or to human serum albumin (HSA) at the same concentration and incubated for 30 minutes at 37° C. Subsequently, 100 μ L of this mixture was added to wells coated with C1-INH, and the presence of IgG antibody bound to the plates was tested, as described for the detection of IgG anti-C1-INH in legend for Figure 1.

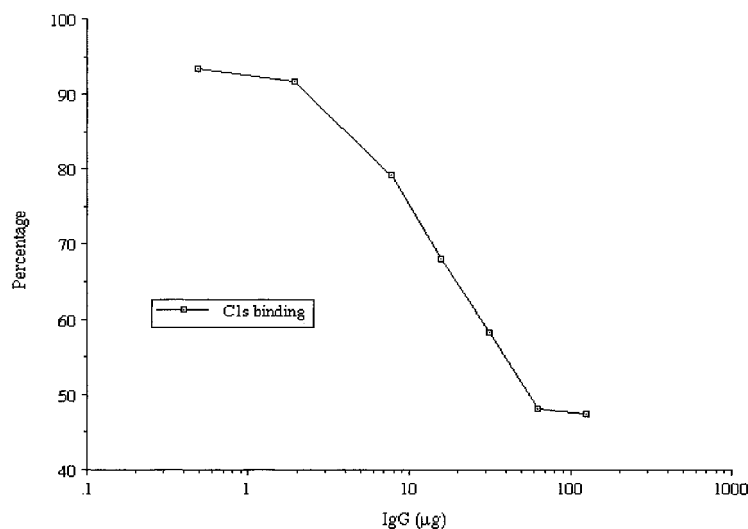


Figure 4. Dose-dependent competition with C1s. A variable amount of purified IgG (0.488 to 125 μ g) was added to the plates coated with C1-INH and incubated for 30 minutes at 37° C. After washing, a constant amount of C1s-biotin (0.14 pmol) was added to each well, incubated for 30 minutes at 37° C, and washed again. Afterward, streptavidin-poly-horseradish peroxidase was added, followed by the addition of an appropriate substrate for peroxidase.

human IgG (Nordic Immunologic Laboratories, Tilburg, the Netherlands), followed by the addition of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (Boehringer Mannheim, Marburg, Germany) as substrate. The percentage of

inhibition was established by comparison with the binding of IgG to the C1-INH-coated plates in the absence of soluble C1-INH.

Inhibition of C1s binding to immobilized C1-INH induced by C1-INH

autoantibody. Microtiter wells, previously coated with C1-INH (200 ng/well), were incubated for 30 minutes at 37° C with several dilutions of the patient's serum (IgG fraction) in PBS containing 0.1% BSA. After washing, a fixed amount of biotinylated C1s (0.14 pmol) was added and incubated for 30 minutes at 37° C. Subsequently, the plate was washed and, in successive steps, streptavidin-poly-horseradish peroxidase (CLB Biotechnology) and an appropriate substrate were added. The percentage of inhibition was calculated by comparison with the binding of C1s in the absence of antibody.

Detection of Truncated C1-INH by Immunoblotting

Plasma samples from patients with type I hereditary angioedema and from normal volunteers were used as controls. Purified C1-INH was also included as a control. Further, one lane contained molecular weight standards (Rainbow, Amersham Pharmacia Biotech, Buckinghamshire, UK). A sample of plasma (1 μ L) containing approximately 25 ng of C1-INH was evaluated in 8% SDS-PAGE under nonreducing conditions, transferred to a polyvinylene difluoride membrane (Immobilon-P, Millipore, Bedford, MA), and saturated for nonspecific binding by incubation overnight with PBS-containing 0.5% nonfat milk (Fluka, Buchs, Switzerland). The presence of species of different molecular weights was investigated with a mouse monoclonal anti-C1-INH antibody¹⁰ that recognizes all forms of C1-INH (native, complexed, and cleaved). The membrane was incubated at room temperature and shaken with this monoclonal anti-human C1-INH for 1 hour, after which a coupled alkaline phosphatase goat antibody to mouse IgG (Pierce, Rockford, IL) was added and incubated for 1 hour at room temperature. The reaction was developed with nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate (Sigma) as substrate. The color reaction was stopped at appropriate times with water.

RESULTS

The presence of polyclonal antibodies to C1-INH of isotypes IgG and IgM (lower intensity) detected by indirect ELISA is shown in Figure 1. Neither IgE nor IgA autoantibodies were detected.

We verified by ELISA that both κ and λ chains were present in the immunoglobulins responsible for the antibody activity (Fig 2). We detected the presence of both κ and λ light chains in the Igs involved in the anti-C1-INH response. In addition, we observed a polyclonal distribution of purified IgG by isoelectric focusing, ranging from isoelectric points 6 to 8 (data not shown).

When we analyzed lymphocyte subpopulations, CD3, CD4, CD8, and CD19 subsets were in the normal range (data not shown). Further, we observed that the percentage of B-lymphocytes (CD19) was 7%, and the κ and λ percentages were 5% and 2%, respectively. These findings are in concordance with a polyclonal origin for this antibody.

IgG was purified from serum by affinity chromatography, and several experiments of competition were performed. In the competitive ELISA, IgG antibody in the presence of soluble C1-INH competed for binding to the immobilized C1-INH, as shown in Figure 3. The inhibition rate reached 52% when 50 nmol soluble C1-INH was added.

Further, we demonstrated that the patient's plasma contained autoantibodies directed against the active center of C1-INH, inasmuch as the addition of several IgG fraction dilutions interfered with the binding of C1s to the C1-INH coated on the ELISA plates (Fig 4). A steric effect could also be involved, but the antibodies did not recognize coated C1-INH from an old batch, which was unable to bind C1s. These facts suggest the requirement of the integrity of the active center for the reactivity with the antibodies. Inhibition percentages ranged from 45 to 95% when several amounts of IgG (0.488 to 125 μ g) were added to the plates coated with C1-INH.

The presence of a C1-INH 96-kD molecular weight form was detected in the plasma of this patient. The analysis

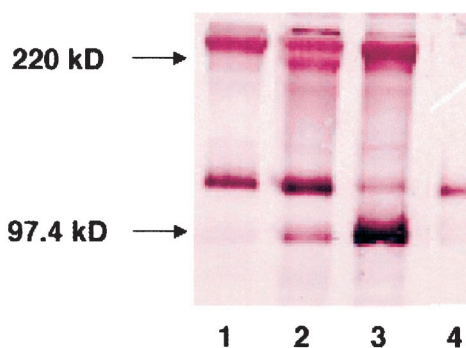


Figure 5. Results of immunoblotting studies, showing presence of 96-kD C1-INH form in plasma from patient with acquired angioedema. Plasma collected in the presence of protease inhibitors was analyzed on 8% SDS-PAGE. Samples were transferred to the polyvinylene difluoride membrane, which was probed with mouse anti-C1-INH monoclonal antibody. Lane 1, normal human plasma. Lane 2, plasma from a patient with type I hereditary angioedema. Lane 3, plasma from patient with acquired angioedema. Lane 4, purified human C1-INH.

of C1-INH in plasma obtained from normal volunteers and a patient with type I C1-INH deficiency revealed C1-INH antigen in distinct bands with different molecular weights (Fig 5). The predominant form of C1-INH in normal plasma samples had an apparent molecular weight of 105 kD (lane 1). Plasma samples obtained from patients with type I hereditary angioedema contained a smaller C1-INH form that had an apparent molecular weight of 96 kD (lane 2). In the plasma sample from the patient with acquired angioedema (lane 3), most of the C1-INH molecule had a molecular weight of 96 kD rather than 105 kD. Lane 4 contained a sample of purified C1-INH (Berinert P).

DISCUSSION

We describe a patient with angioedema attributable to acquired C1-INH deficiency, as evidenced by complement levels and the presence of anti-C1-INH autoantibodies. The autoantibodies were polyclonal, inasmuch as both IgM and IgG autoantibodies were present, and these antibodies contained both κ and λ chains. Further, no evidence of paraprotein was detected by either capillary electrophoresis or immunoelectrophoresis. Isoelectric focusing of purified IgG disclosed a polyclonal distribution of IgG. Further, the percentage of B-lymphocytes was in the normal range, and the κ -to- λ ratio corresponded to a polyclonal dis-

tribution. These autoantibodies were able to show competition between soluble and immobilized C1-INH and to impede the binding to C1s when serially diluted antibodies were added to the coated plates with C1-INH.

By immunoblotting, we detected a 96-kD C1-INH with a molecular weight lower than normal C1-INH (105 kD). This smaller form was previously described in this type of angioedema.¹¹⁻¹³ The origin of this form is a debated issue. This smaller C1-INH form could be generated by the action of proteases because investigators have observed *in vitro* that modified inactive inhibitor may be generated with an apparent molecular weight lower than that for native inhibitor during the interaction of C1-INH with target protease.^{14,15} Malbran et al¹² proposed that anti-C1-INH autoantibodies do not interfere with the initial interaction between C1-INH and its target proteases (C1r, C1s, kallikrein, activated Hageman factor, and plasmin) but rather destabilize their interaction and thereby impair complex formation. This mechanism could account for the lack of protease-C1-INH complexes in the plasma of patients with acquired C1-INH deficiency and anti-C1-INH autoantibodies.^{12,16} Further, this 96-kD form is thought to be generated by the association of autoantibodies with circulating uncleaved C1-INH; hence, cleavage of C1-INH by C1s can en-

sue.¹⁷ Thus, the autoantibodies convert C1-INH from an inhibitor to a substrate. After cleavage of C1-INH by C1s in the presence of anti-C1-INH autoantibody, the epitope is lost because the antibody no longer binds to the cleaved C1-INH molecule.

Followup of the anti-C1-INH autoantibodies showed no significant changes during 2 years, and in agreement with this finding, C1-INH, C4, and C1q remained decreased during this period. Treatment with corticosteroids had been inefficient in stopping the attacks of angioedema. Further, when the patient was treated with androgens, the clinical situation worsened; in contrast, antifibrinolytic therapy improved his clinical status. This relationship had been observed previously.^{18,19} With intravenous administration of C1-INH, the acute episodes of angioedema resolved. In acquired angioedema, alternative treatments such as plasmapheresis and cytotoxic therapy have also been used.²⁰

CONCLUSION

We describe a patient who had acquired angioedema with isolated anti-C1-INH antibodies in association with the presence of a 96-kD form of inhibitor. Several treatment options were ineffective. A good response was attained when antifibrinolytic agents were administered as long-term prophylaxis, and the acute episodes of angioedema resolved with intravenous administration of C1-INH.

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