

Immunodeficiency and other clinical immunology

C1-inhibitor binding monoclonal immunoglobulins in three patients with acquired angioneurotic edema

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The syndrome of acquired angioneurotic edema (AAE) is characterized by the adult onset of angioedema, the lack of evidence for inheritance of the disorder, and the frequent association of the C1-inhibitor (C1-INH) deficiency with lymphoproliferative or other malignant diseases. Recently, a new type of AAE (type II AAE) has been described. The two major biologic differences of this new syndrome compared with all other previously reported AAE cases (type I AAE) are the presence in patients' sera of both anti-C1-INH autoantibodies, often monoclonal, and a circulating low molecular weight (95 kd) C1-INH protein. From the clinical point of view, the absence of underlying lymphoproliferative disease is the hallmark of type II AAE compared with type I AAE. However, the distinction between type I and type II AAE may not be so clear-cut. We report three patients with monoclonal gammopathies and AAE for whom the initial diagnosis was type I AAE. The demonstration by ELISA of the C1-INH binding ability of their monoclonal immunoglobulins in addition to the presence of 95 kd C1-INH protein enables us to change the diagnosis to type II AAE. From the therapeutic point of view, it is crucial to detect the anti-C1-INH antibody and to analyze the C1-INH size to distinguish type I and type II AAE, especially if patients have a monoclonal gammopathy, to give the appropriate treatment (attenuated androgens vs immunosuppressive regimen, respectively) to prevent a fatal outcome. (J ALLERGY CLIN IMMUNOL 1996;97:998-1008.)

Key words: C1-inhibitor deficiency, acquired angioneurotic edema

Human complement C1-inhibitor (C1-INH) is a serine proteinase inhibitor with a molecular weight of 105 kd¹ that belongs to the serpin family. It inactivates the C1r and C1s components of the complement system and enzymes of the coagula-

Abbreviations used

AAE:	Acquired angioneurotic edema
C1-INH:	C1-inhibitor
CLL:	Chronic lymphocytic leukemia
ESR:	Erythrocyte sedimentation rate
HAE:	Hereditary angioneurotic edema
IEP:	Immunoelectrophoresis
NHS:	Normal human serum
PBS:	Phosphate-buffered saline

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tion (factors XIa, XIIa and XIIIf), fibrinolytic (plasmin), and kinin systems (kallikrein).² It is therefore a major inhibitor of the inflammatory response.

Binding of the target protease to C1-INH leads to cleavage of the peptide bond between arginine

444 and threonine 445 in the reactive center. This results in the formation of a stable acyl-enzyme complex in which the protease is inactivated.

A genetic defect of C1-INH, inherited in an autosomal dominant manner, was recognized in 1969³ as the molecular basis of the hereditary angioneurotic edema (HAE), first described by Osler⁴ at the end of the last century. The clinical manifestations include swelling of the skin or mucosa with preferential involvement of the extremities, face, gastrointestinal tract, and upper airway.⁵⁻⁷

Determination of C1-INH protein level and function in plasma have defined two major forms of HAE. In type I HAE, both the C1-INH protein and functional activity are decreased by 5% to 30% of normal by impaired biosynthesis and increased catabolic rate.⁸ In type II HAE, the C1-INH level is normal or elevated, but C1-INH function is decreased because of the synthesis of dysfunctional C1-INH species.^{9,10} The underlying molecular changes are of two types: mainly mutations in the reactive center (exon VIII) and sometimes mutations outside it.¹¹ This leads to abnormal folding of the C1-INH so that the reactive center is unavailable for appropriate interactions with the target protease. The latter is therefore not inhibited but still able to cleave to the C1-INH molecule.

In 1972, Caldwell et al.¹² reported the first recognized case of acquired angioneurotic edema (AAE). Since then, about 30 cases have been reported.^{13,14} AAE syndrome is characterized by adult onset of angioedema, lack of evidence for inheritance of the disorder, and frequent association of the C1-INH deficiency with lymphoproliferative or other malignant diseases: non-Hodgkin's B-cell lymphoma,¹⁵⁻²¹ T-cell lymphoma,²² chronic lymphocytic leukemia (CLL),²³⁻²⁵ Waldenström's macroglobulinemia,²⁶ monoclonal gammopathy,²⁷⁻³⁰ myelofibrosis,³¹ and rectal carcinoma.³² The clinical symptoms of both syndromes are indistinguishable. From the biologic point of view, the main difference between AAE and HAE is, in the former, the depressed levels of C1 in addition to the low levels of C2 and C4 also observed in the latter. This results from an accelerated catabolism of a normally synthesized C1-INH molecule³³ by either plasmatic or cellular complement-activating factors that could be either a monoclonal 7S IgM or idiotype-anti-idiotype immune complexes.³⁴⁻³⁹

Recently, a new type of AAE (type II AAE) has been described.⁴⁰⁻⁴⁷ The two major biologic differences of this new syndrome compared with all other previously reported AAE cases (type I AAE)

are the presence in patients' sera of both anti-C1-INH autoantibodies, often monoclonal, and a circulating low molecular weight (95 kd) C1-INH protein. In contrast to type I AAE, the hallmark of type II AAE is the absence of underlying disease. In type I AAE the underlying malignancies produce complement-activating factors, idiotype-anti-idiotype, or other immune complexes that destroy C1-INH function. In type II AAE, however, the autoantibody seems to interfere with the inactivation of the activated ligated C1s by C1-INH. This leads to an unopposed C1 activation (by the non-inhibited protease), which has a biologic profile similar to type II HAE with a resulting functionally inactive 95 kd cleaved C1-INH.^{46, 48, 49}

However, the distinction between type I and type II AAE may not be so clear-cut. We report three patients with monoclonal gammopathies and AAE for whom the initial diagnosis was type I AAE. The demonstration by ELISA of the C1-INH binding ability of these monoclonal immunoglobulins enables us to change the diagnosis to type II AAE. The therapeutic implications of such a distinction are discussed.

CASE REPORTS

Patient 1

In May 1977, a 55-year-old man had symptoms of anemia, splenomegaly, and lymphadenopathy. The day before admission, the patient complained of an acute abdominal pain. At the physical examination on admission, a huge spleen and slight bilateral axillary and inguinal lymphadenopathies were noticed. No other clinical abnormality was observed except for pallor. Hepatomegaly was verified by ultrasonogram, and retroperitoneal lymphadenopathy was demonstrated by lymphogram. Peripheral blood count at that time showed hemoglobin, 9.6 gm/dl; reticulocytes, $29 \times 10^9/L$; leukocytes, $11.3 \times 10^9/L$ with 73% lymphocytes; platelets, $119 \times 10^9/L$. Morphologic examination of the bone marrow showed a decrease in normal hematopoiesis; about 60% of the cells were small, mature-looking lymphocytes accompanied by 2% lymphoplasmacytic cells. The patient was diagnosed as having CLL. The erythrocyte sedimentation rate (ESR) was 52/95 mm. His biochemical profile was within normal limits, except for calcemia (86 mg/L). Electrophoresis showed two distinct abnormal bands in the gamma and beta regions. The immunoelectrophoresis (IEP) confirmed the biclonal gammopathy with the presence of two monoclonal Igs, an IgM- κ and an IgA- κ . Normal IgG proteins were decreased. Urine profile showed IgA κ M-component. Spleen irradiation (300 cGY) was performed. Two weeks later, administration of chlorambucil, 4 mg daily, was started and maintained until August 1978, then replaced by administration of cyclophosphamide, 25 mg

daily. Nevertheless, the patient experienced a dramatic recurrent upper airway angioedema with cardiopulmonary arrest that required a tracheostomy and resuscitation. A permanent tracheal cannula was left in place for 6 years to prevent recurrence of laryngeal edema. Another laryngeal attack occurred shortly afterward. CLL was controlled by continuous therapy with alkylating agent. As measured by radial immunodiffusion, the C4 level was depressed to less than 0.04 gm/L between May 1979 and March 1982, whereas it was initially 0.009 gm/L (normal, 0.22 to 0.50 gm/L). By the same method, C1-INH level was measured at 0.152 gm/L (normal, 0.23 to 0.30 gm/L); the functional level, measured by an enzymatic method,⁵⁰ was also decreased to 0.40 U/min/ml (normal, 5 ± 2.5 U/min/ml). There was no familial history of angioedema. These results were consistent with AAE. From that time, the patient received 400 mg of danazol daily without any improvement. However, after 3 years of chemotherapeutic maintenance and despite several attacks of acute dyspnea treated with cortisone, no sign of CLL relapse was observed and the patient was relatively well. In April 1980, IEP showed that the IgM- κ persisted, whereas the IgA- κ was slightly reduced, suggesting a link between this reduction and the parallel improvement of the clinical status—notably the absence of recurrent attacks of angioedema—despite a low level of C1-INH (0.048 gm/L). Results of clinical examination were normal. The patient was considered to be in remission, and oral chemotherapy was discontinued.

He was again referred to the hospital in December 1989 for evaluation of a left cervical lymphadenopathy. Physical examination also revealed left axillary and bilateral inguinal lymph node enlargement. During abdominal palpation, neither splenomegaly nor hepatomegaly was noted. Nevertheless, thoracoabdominal tomogram revealed slight spleen enlargement and low thoracic and retroperitoneal lymphadenopathy. His blood count was normal. A bone marrow biopsy specimen showed an infiltration of lymphoplasmacytic cells. The same picture was observed in a laterocervical biopsied lymph node, leading to the diagnosis of lymphoplasmacytic lymphoma. Paradoxically, C1-INH level, as measured by nephelometry, was normal. Serum IEP showed that the IgM- κ persisted. In contrast, the IgA- κ was practically undetectable. Three cycles of cyclophosphamide, hydroxyrubicin, oncovin, and prednisolone were administered without any benefit; the volume of lymphadenopathy remained unchanged. Cardiac rhythm disturbances contraindicated the continued use of adriamycin. Treatment with 100 mg of oral etoposide daily and 100 mg of cyclophosphamide daily, each for 5 days and two courses monthly, was started in April 1990. His course was complicated in June by a pneumococcal meningitis and septicemia. At that time, a bone marrow aspirate showed a normal cellularity accounting for 15% lymphocytes and 2% lymphoplasmacytic cells. In October 1990, the patient was treated again

for abdominal discomfort. This symptom corresponded to physical examination to the presence of bulky masses accompanied by compressive bilateral edema of lower limbs, which suggested an aggressive evolution leading to a new chemotherapeutic treatment with a variant of Pro Mace MOPP regimen (bleomycin and cytosine arabinoside in place of methotrexate and adriamycin, respectively) administered sequentially between November 1990 and March 1991. Meanwhile, the reduction of abdominal lymphadenopathy was remarkable. However, shortly thereafter, the patient died of bilateral interstitial pneumonia.

Patient 2

A 75-year-old man with recurrent attacks of hand swelling once a month for 1 year experienced the onset of laryngeal edema in January 1989. The upper airway obstruction improved with intravenous administration of high-dose prednisone. On physical examination neither hepatosplenomegaly nor enlargement of lymph nodes was noted. There was no history of bone pain or bleeding. ESR was 7 mm/hr; hematocrit, 45%; white blood cell count, $8.9 \times 10^9/L$ with 80% neutrophils. In the absence of familial history of angioedema and in view of the late onset of the illness, a diagnosis of presumed AAE was made based on the following values: normal C3 at 0.82 gm/L (0.55 to 1.20 gm/L), decreased C4 at 0.024 gm/L (0.20 to 0.50 gm/L), and C1-INH at 0.059 gm/L (0.15 to 0.25 gm/L, by nephelometry) with no functional complement activity ($CH_{50} < 5\%$). IEP disclosed a monoclonal IgM- κ with normal residual polyclonal immunoglobulins. Treatment with danazol was started, and the patient was discharged. He suddenly died at home of laryngeal edema just before the scheduled hospitalization in the hematology unit to investigate his underlying lymphoproliferation.

Patient 3

An 83-year-old woman complained in July 1990 of six to eight episodic, recurrent, nonpitting self-limited attacks of face swelling not associated with urticaria or pruritus. The cutaneous angioedema started in February, was accompanied by neither colicky pains nor laryngeal edema, and was responsive to intravenous corticoid treatment. There was no familial history of episodic cutaneous angioedema. In view of the late onset of the patient's illness, a diagnosis of AAE was suspected on the basis of the following data: decreased C4 (< 0.03 gm/L) and C1-INH levels (0.076 gm/L) with normal C3 (0.88 gm/L). There was no abnormality on physical examination. ESR was 15 mm/hour; hemoglobin, 14.7 gm/dl; white blood cell count, $13.4 \times 10^9/L$ with 77.6% neutrophils. IEP disclosed a monoclonal IgA- κ with normal level of IgG and a slight polyclonal increase of IgM. The patient refused further investigation. She had one or two episodic attacks of cutaneous swelling per year, which responded well to corticoid treatment. She

TABLE 1. Laboratory data for the three patients with AAE and monoclonal gammopathy

Patient	Date	Monoclonal Ig	IgG (gm/L)	IgM (gm/L)	IgA (gm/L)	C1q (%)	C3 (gm/L)	C4 (gm/L)	CH ₅₀ (%)	C1-INH	
										gm/L	kd(%)*
1	1979	IgM-κ	5.79	4.11	6.06	<12	0.78	0.04	<5	0.152	95 (100%)
1	1990	IgA-κ									
		IgM-κ	9.46	25.6	0.44	98	0.42	0.13	100	0.12	105 (30%) 95 (70%)
2	1989	IgM-κ	10.5	1.86	3.78	14	0.82	0.02	<5	0.059	95 (100%)
3	1990	IgA-κ	9.37	4.46	4.94	<10	0.88	<0.03	<5	0.076	95 (100%)
3	1995	IgA-κ	9.47	4.11	5.21	80	0.85	<0.03	<5	0.18	95 (100%)
Normal	maxima		18	4.5	2.8	116	1.2	0.5	120	0.42	105 kd
	minima		8	0.9	0.6	84	0.55	0.2	80	0.26	

IgG, IgA, IgM, C3, C4 and C1-INH: nephelometry; C1q: radial immunodiffusion.

*Serum: except plasma for patient 3, 1995.

received neither immunosuppressive nor attenuated androgen therapy until February 1995. At that time she still had AAE with normal C3 (0.85 gm/L), decreased C4 (<0.03 gm/L), slightly decreased C1-INH levels (0.18 gm/L) with no functional complement activity (CH₅₀ <5%), and the same IEP pattern with monoclonal IgA-κ with slightly polyclonally increased IgM.

METHODS

Immunologic assessment

Serum samples from the patients were stored at -80° C within 6 hours of collection and were thawed once before assay. Immunoglobulin and complement component levels were determined by immunonephelometry with monospecific antisera (IgA, IgG, IgM, C3, C4, C1-INH) (BNA; Behring, Rueil-Malmaison, France). The typing of monoclonal Ig was carried out with polyspecific and monospecific sera (Silenius, Hawthorn, Australia) by IEP according to the micro method described by Scheidegger.⁵¹ The functional activity of the classical pathway was measured by the classical CH₅₀ assay,⁵² testing the ability of serum to lyse antibody-coated sheep erythrocytes. Autoantibodies were detected by indirect immunofluorescence on rat liver, stomach, and kidney sections with a fluorescein isothiocyanate-labeled goat anti-whole human immunoglobulins antiserum (Pasteur Institute, Paris, France). C1q was determined by radial immunodiffusion with a monospecific anti-C1q antiserum (Behring).

C1-INH-binding ELISA

To characterize a possible C1-INH-binding ability of the monoclonal Igs, a C1-INH-binding ELISA was set up. C1-INH was purified as previously described.⁵³ The optimal concentration of purified C1-INH for coating the ELISA plates was determined to be 1 µg/ml in 0.2 mol/L borate-buffered saline (pH 8.2) in preliminary checkerboard experiments. C1-INH was coated to the polyvinylchloride microtiter plates (CML, Nemours, France), 100 µl per well, at 4° C overnight. After three washes with phosphate buffered saline (PBS) (pH 7.4)

containing 0.1% Tween-20, the wells were saturated with PBS-Tween containing 1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) for 2 hours at room temperature. After five washes, sera were diluted 500-fold in PBS-Tween and incubated in triplicate for 1 hour at 37° C. After five washes, 100 µl of serially diluted horseradish peroxidase conjugate were incubated for 1 hour at 37° C. Preliminary experiments showed that the optimal dilutions for each conjugate were: anti-human IgG, IgA, IgM, kappa, and lambda (Dakopatts, Glaxtrup, Denmark) = 1:2000; anti-human IgG (The Binding Site Ltd., Birmingham, England) = 1:6000; anti-human IgA (The Binding Site Ltd.) = 1:4000; and anti-human IgM (The Binding Site Ltd.) = 1:2000. After five washes, 100 µl of substrate (o-phenylenediamine dihydrochloride, 12.8 mg in 5 ml of 0.05 mol/L citrate-phosphate buffer [pH 5.0] containing 0.02% H₂O₂) was added and incubated for 10 minutes at 37° C. The absorption was measured at 492 nm.

Dilution curves. Plates were coated with C1-INH at a concentration of 1 µg/ml. One AAE serum, one normal human serum, and one Ig-deficient serum were tested at dilutions ranging from 1:10 to 1:50,000 in the ELISA.

To exclude the hypothesis that C1-INH-binding activity in patients' sera was mediated by immune complexes containing the C1r and C1s C1 components able to bind to the coated C1-INH, the same dilution experiment was performed with the AAE serum in the presence of 40 mmol/L ethylenediaminetetraacetic acid, which dissociates C1 by chelating calcium.

Western blot analysis of C1-inhibitor

C1s was purified according to the method of Arlaud et al.⁵⁴ To analyze the C1-INH in patient plasma (or serum), plasma (or serum) incubated or not with purified C1s (molecular ratio between C1-INH present in plasma and exogenous C1s:1:1) was diluted in sodium dodecylsulfate sample buffer (0.1 mol/L Tris-HCl, 1% sodium dodecylsulfate, 4 mol/L urea, pH = 8.0) and heated at 100° C for 5 minutes. Then, diluted plasma (or

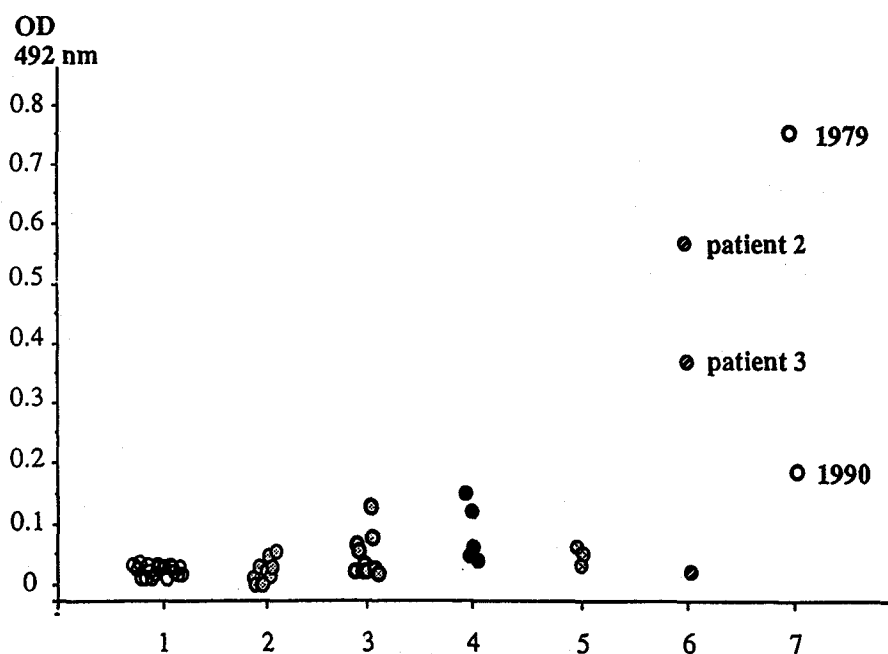


FIG. 1. C1-INH ELISA. The ELISA was performed as described in Methods. Horseradish peroxidase conjugate: anti-human Igs (G + A + M) (Dakopatts); dilution, 1:2000. 1, NHS ($n = 20$); 2, monoclonal IgM without AAE ($n = 10$); 3, monoclonal IgA without AAE ($n = 10$); 4, biclonal gammopathy (IgM + IgA) without AAE ($n = 5$); 5, HAE ($n = 3$); 6, AAE with ($n = 2$) or without ($n = 1$) monoclonal gammopathy; 7, patient 1 with biclonal (1979) or monoclonal (1990) gammopathy.

serum) was loaded on a 7.5% polyacrylamide gel to load 12 ng of C1-INH protein.

After electrophoresis, proteins were transferred onto Immobilon polyvinylidene fluoride membranes. After blocking with 0.1% Tween 20 in Tris-buffered saline, the membranes were incubated directly with goat anti-human C1-INH peroxidase conjugate (The Binding Site Ltd.) and developed with α -chloronaphthol.

RESULTS

Immunologic assessment

All three patients had the biologic profile of AAE (Table I): late onset of angioedema (namely at 55, 75, and 83 years of age, respectively) and absence of familial history of such illness. The C3 level was normal; whereas C4, C1-INH, and C1q levels were greatly diminished. For patient 1, all these parameters except C1-INH returned to normal levels in 1990 when his serum monoclonal IgA was no longer detectable (Table I). Common autoantibodies were not detected in sera of the three patients.

C1-INH-binding ELISA

In our assay only the three patients with AAE and monoclonal gammopathy demonstrated a positive signal statistically different from the 20 nor-

mal healthy blood donors (Fig. 1). Control patients with monoclonal IgM ($n = 10$), IgA ($n = 10$), or both ($n = 5$) but without angioedema had negative results. The same was true for the three tested patients with HAE and for the patient with AAE and no monoclonal gammopathy.

The dose-dependent response for the initial serum of patient 1 is shown in Fig. 2 compared with a normal human serum (NHS) and an Ig-deficient serum sample from a patient with a common variable immunodeficiency. The sera of patients with either biclonal gammopathy ($n = 3$), monoclonal IgA ($n = 4$), or IgM ($n = 1$) whose sera had an optical density between 0.05 and 0.15 (Fig. 1) (i.e., more than the mean plus 3 standard deviations of the normal blood donors, $0.024 + 3 \times 0.008$) have such an increased protein level that these 500-fold diluted sera have more Ig quantity than the 50-fold diluted NHS, which has an optical density of 0.18.

The binding did not require divalent cation because the response was identical in the presence of 40 mmol/L ethylenediaminetetraacetic acid. This fact rules out the binding of preformed idiotype-anti-idiotype immune complexes activating the classical pathway and bearing the C1 subcom-

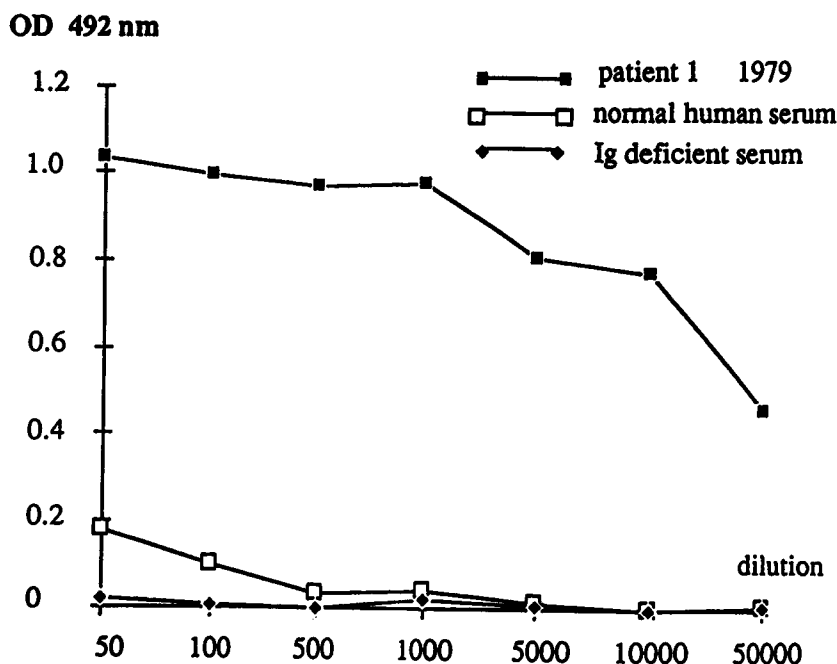


FIG. 2. C1-INH ELISA dose dependence of binding. The ELISA was performed with diluted serum as described in Methods. NHS: IgG = 12.1 gm/L; IgA = 2.56 gm/L; IgM = 1.80 gm/L. Ig-deficient serum: IgG = 0.45 gm/L; IgA = 0.0036 gm/L; IgM = 0.23 gm/L.

ponents C1r and C1s able to ligate the coated C1-INH (Fig. 3).

Finally, isotype analysis of the C1-INH-binding Ig (Fig. 4) clearly showed that for patient 3, IgA was the only Ig able to bind to C1-INH in accordance with the nature of the monoclonal Ig found in her serum either in 1990 or in 1995. For patient 1, the major isotype was alpha, both in the first serum sample with biclonal gammopathy and in the second sample with no more detectable monoclonal IgA. For patient 2, with monoclonal IgM, mixed IgM and IgG binding was observed.

Western blot analysis of C1-INH

C1-INH size studies were performed on plasma from patient 3 in 1995. For the older blood samples, only frozen sera were available (Table I, Fig. 5). In 1995 all the C1-INH molecules in the plasma of patient 3 behaved as a 95 kd cleaved nonfunctional protein, unable to form stable complexes with C1s (Fig. 5). The same was true for her serum in 1990 (Table I), as well as for the serum of patient 2. This feature was also the case for the first serum of patient 1, whereas up to 30% of C1-INH was in the native 105 kd form in his second serum sample, which contained only low amounts of monoclonal IgM (Table I).

DISCUSSION

In this study we describe three adult patients showing C1-INH-binding monoclonal Ig associated with symptoms of angioedema. The diagnosis of C1-INH deficiency was made on the basis of the absence of hemolytic activity as measured by the CH₅₀ assay and the depressed levels of C4 and C1-INH. The diagnosis of AAE was based on the following data, similar to previously reported cases^{55,56}: late occurrence of the illness in adulthood with no family history and diminished level of C1q. Until now, only one patient with AAE and a normal level of C1 has been reported.⁵⁷ For patient 3, at the last examination in 1995, C1q level was only slightly decreased (80%, normal = 84% to 116%), even though she still had clinical and biologic AAE. The possibility that she has a genetic defect is unlikely. Indeed, her symptoms appeared when she was 83, C1q was greatly diminished the first time, and she had no family history. So we confirm that autoimmune AAE may actually occur with normal C1q plasma levels.

In one of our patients with a 14-year follow-up, a well-documented lymphoproliferative disorder, namely a CLL secondarily evolving into a lymphoplasmacytic lymphoma, was associated with the AAE. In the other two patients there were no clinical manifestations of hematologic disorder

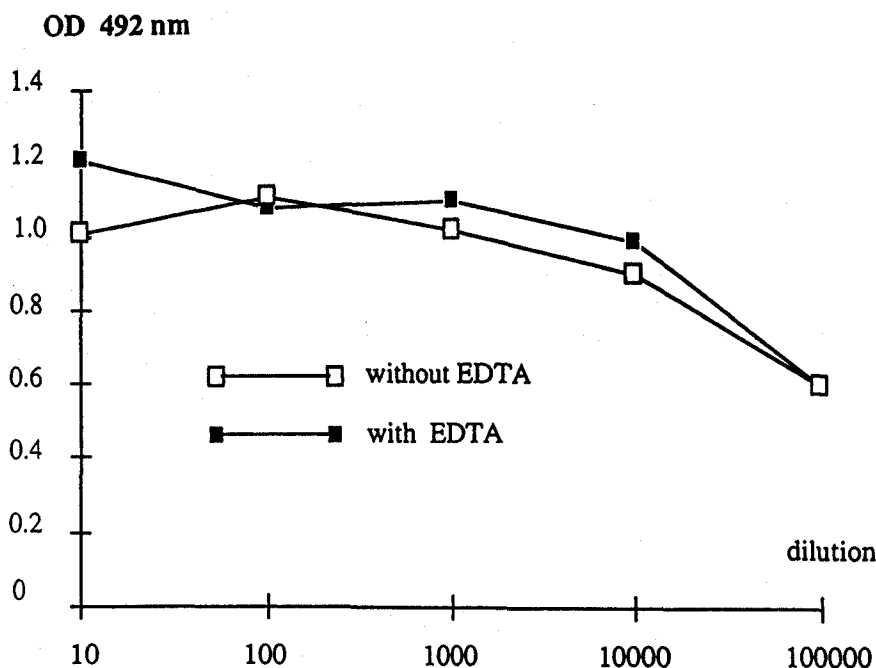


FIG. 3. C1-INH ELISA: effect of divalent cation chelating agent. Same experiment as shown in Fig. 2 performed with serum of patient 1 from 1979 with (40 mmol/L) or without ethylenediaminetetraacetic acid (EDTA).

such as spleen, liver, or lymph node enlargement. For material reasons, no extensive investigation could be performed to identify an underlying disease responsible for the synthesis of the monoclonal serum Ig.

At first sight and according to the recently reviewed⁵⁵ description of the two types of AAE, the clinical data (association with a lymphoproliferative disorder or at least with a monoclonal gammopathy) favored the diagnosis of type I AAE. In this disorder,¹³ depressed functional C1-INH activity results from a massive consumption secondary to complement classical pathway activation by either cryoglobulin⁵⁸⁻⁶⁰ or immune complexes of hematologic,³⁷ autoimmune,^{61,62} or infectious origins.⁶³

However, clinical and biologic data argued against this mechanism and prompted us to search for anti-C1-INH autoantibodies, hallmarks of type II AAE.^{47,55} In this syndrome the anti-C1-INH autoantibody binds to the normally synthesized C1-INH and modifies the interactions with its target proteases, converting the C1-INH molecule from an inhibitor to a normal substrate of the protease. It does not usually prevent the first step (binding of the protease to C1-INH with subsequent cleavage of the latter) but interferes with the second step (inactivation of the protease) by destabilizing the enzyme-inhibitor complex leading to appearance of enzyme-cleaved

inactive 95 kd C1-INH in patients' plasma.⁴⁸ To date, only one patient with type II AAE and oligoclonal anti-C1-INH autoantibody has been reported as having C1-INH of normal molecular size and an autoantibody directed against the reactive center of the C1-INH molecule.⁶⁴ We hypothesize that this peculiar autoantibody could interfere with the first step of the interaction between the C1-INH and its target protease as sustained by its normal fractional catabolic rate in contrast with the increased rate observed when cleavage at the reactive center occurs as in all the other reported cases of type II AAE.

For our patients 1 and 2, the use of attenuated androgens, namely danazol, was of no benefit and could not prevent a fatal outcome caused by upper airway edema (patient 2). The efficacy of this drug, by increasing C1-INH liver synthesis, is well documented in the two types of HAE and in type I AAE.^{7,65-69} In contrast, for patients with type II AAE, these drugs are not effective at all^{42,70} or the disease becomes refractory to the treatment during follow-up.^{40,57,68,71} This reaction is probably caused by the large excess of autoantibody able to neutralize any increased C1-INH induced by the androgens.⁵⁵ This could also explain the partial or complete resistance^{41,42,57} or even the exacerbation⁷² of angioedema attacks after the infusion of either fresh frozen plasma or C1-INH concentrate.

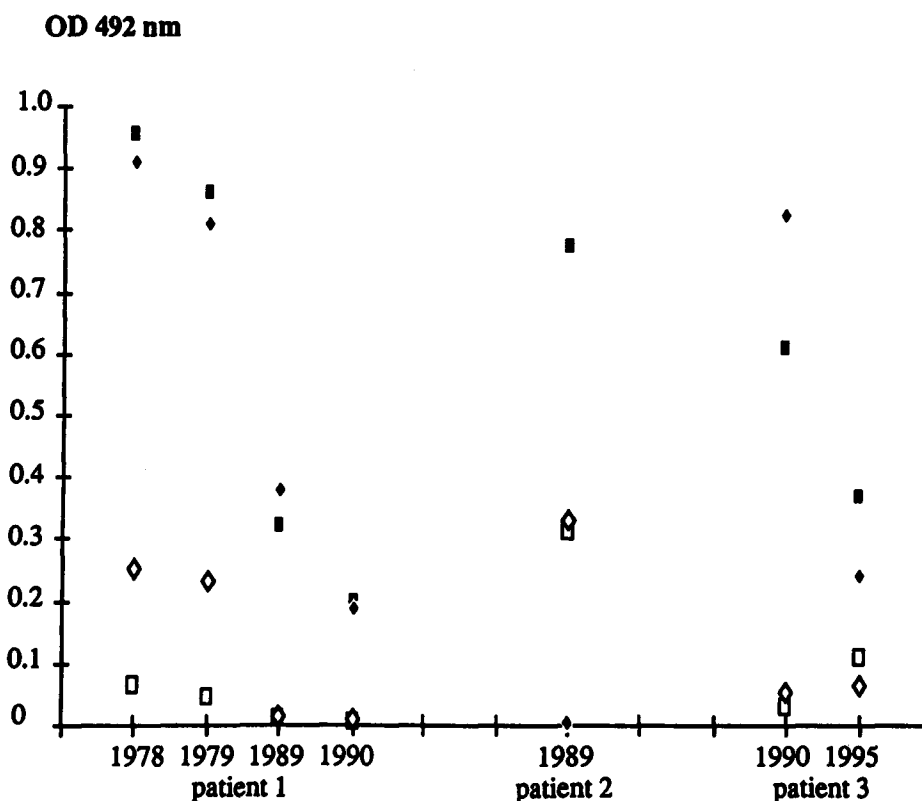


FIG. 4. C1-INH ELISA: isotype analysis. Same experiment as Fig. 1. Horseradish peroxidase conjugates: ■, anti-human Igs (G + A + M) (Dakopatts), dilution 1:2000; ◆, anti-human α (binding site), dilution 1:4000; ◇, anti-human μ (Binding Site), dilution 1:2000; □, anti-human γ (Binding Site), dilution 1:6000.

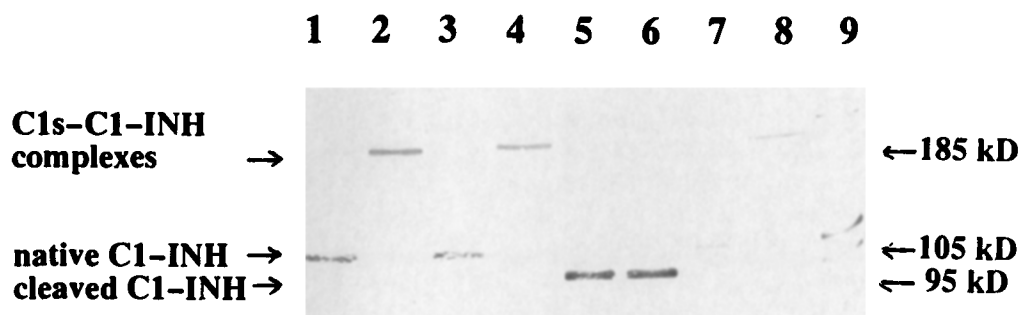


FIG. 5. C1-INH Western blot analysis. The C1-INH molecular size in plasma was studied by Western blot as described in Methods. Lane 1, pooled normal human plasma; lane 2, pooled normal human plasma + C1s; lanes 3 and 7, individual normal human plasma; lanes 4 and 8, individual normal human plasma + C1s; lane 5, patient 3 plasma; lane 6, patient 3 plasma + C1s; lane 9, molecular weight markers.

In our C1-INH-binding ELISA only sera of the three patients with AAE and monoclonal Ig were able to bind to the coated C1-INH. Preformed immune complexes, as reported in type I AAE,³⁷ were ruled out by using divalent cation chelating agent, so that the true antibody nature of the

binding seems the most likely. As already reported by Cicardi et al.,⁵⁷ we have confirmed, in our limited sample, the high specificity of the anti-C1-INH antibody for type II AAE, especially for the negative HAE group in which some patients have received plasma or C1-INH concentrate, and its

presence is isolated because no other autoantibodies were detected in sera of our three patients. The autoantibodies were found in all three cases in the absence of any preceding infusion of plasma or C1-INH concentrate, confirming the autoimmune nature of this disorder, as already demonstrated by other authors.^{40, 42, 73} The finding of only cleaved 95 kd C1-INH molecules in patients' blood samples during clinically active AAE was the second biologic argument for making the diagnosis of type II AAE.^{47, 55} Finding this in the plasma of patient 3 in 1995 and the differences between the two serum samples from patient 1 validate the results of the C1-INH molecular size study, ruling out artefactual cleavage after collection.

In the literature these autoantibodies, usually detected by ELISA, are assumed to be of monoclonal origin on the basis of the following data. First, no immune precipitates are seen when purified C1-INH and patients' serum or purified Ig are mixed either in the fluid phase or in gel.^{42, 46} Second, gel filtration studies reveal a molecular weight of approximately 400 kd for the C1-INH-anti-C1-INH complex, consistent with a 1:1 ratio of antigen to antibody.⁴² Third, isoelectric focusing studies show a restricted mobility for the affinity-purified antibody.⁴⁰ Fourth, these antibodies seem highly specific for the binding site of the C1-INH, because they cannot bind to preformed C1-INH-C1s complexes.⁴² Nevertheless, only four patients with anti-C1-INH autoantibodies and serum monoclonal Ig have been reported: two by Cicardi et al.⁵⁷ and two by Zuraw and Chambers.⁷⁴ For the other patients with type II AAE and anti-C1-INH antibodies, no immunoelectrophoretic abnormality was noticed.⁷⁵ One patient of Cicardi⁵⁷ had monoclonal gammopathy of uncertain significance 1 year after the onset of AAE. He has been followed up for 15 years with no evidence of malignancy or increase of the M component. Nevertheless, AAE attacks stopped spontaneously after 5 years. The second patient had Waldenström's macroglobulinemia 3 years after the first attack of AAE, and his anti-C1-INH autoantibody was of the IgG isotype. In the report of Zuraw and Chambers⁷⁴ two of four patients with type II AAE had a monoclonal Ig, whereas of the two patients with autoantibody-negative AAE (type I), one had a monoclonal Ig and the other had CLL. Zuraw and Chambers⁷⁴ confirm the oligoclonal predominance of the anti-C1-INH antibody, its specificity for AAE (no patients with positive HAE or normal control subjects), and its isolated occurrence.

The follow-up of patient 1 is of particular inter-

est. Indeed, angioedema attacks "spontaneously" improved with the concomitant disappearance of one of the monoclonal Igs (IgA- κ). At the same time, anti-C1-INH autoantibody level dramatically decreased but was still of IgA isotype, and 30% of his C1-INH molecules were fully active 105 kd molecules. If this patient had type I AAE with idiotype-anti-idiotype immune complexes, the dramatic decrease of his monoclonal IgA in 1990 could explain a less important activation of the classical pathway. Alternatively, and more probably, if he had type II AAE, the improvement could be explained by the suppressive effect of the cytotoxic chemotherapy on the malignant IgA B-cell clone in such a way that its monoclonal alpha isotype C1-INH-binding monoclonal IgA was produced at a level not detectable by IEP, but still detectable by ELISA, and in amounts too low to inactivate all the normally synthesized C1-INH. This change resulted in a protective effect against angioedema attacks, even with only 30% of fully active C1-INH.

This special follow-up confirms the data of Donaldson et al.⁷⁰ who observed clinical improvement in a patient with type II AAE and anti-C1-INH antibody after plasmapheresis and cytotoxic therapy. In view of the aforementioned pathophysiology, only therapy aimed at decreasing the level of the autoantibody will be of clinical use. Any induced C1-INH increase, either by attenuated androgens or by infusion of plasma or C1-INH concentrate, will be abrogated by the large excess of autoantibody usually present, or may even be harmful by perpetuating the autoantigen challenge. So, from the therapeutic point of view, it is crucial to detect the anti-C1-INH antibody and to analyze the C1-INH size to distinguish type I and type II AAE, especially if patients have a monoclonal gammopathy, to give the appropriate treatment (attenuated androgens vs immunosuppressive regimen, respectively) to prevent a fatal outcome.

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