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J Clin Invest. 1989;83(6):1794-1799. <https://doi.org/10.1172/JCI114084>.

Research Article

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Acquired C1 Inhibitor (C1-INH) Deficiency Type II

Replacement Therapy with C1-INH and Analysis of Patients' C1-INH and Anti-C1-INH Autoantibodies

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Abstract

The response of two patients with autoantibody-mediated C1-inhibitor (C1-INH) deficiency to replacement therapy with C1-INH was studied over a period of 3 d. In patient 1 an acute attack of angioedema was successfully managed by infusion of 1,000 U of C1-INH concentrate. C1-INH function returned to normal levels within 30 min, while CH50 and C4 peaked after 6–7 h and C1 hemolytic activity reached 50–60% of normal after 3 d. Immediately after the injection an increase in C1-INH-anti-C1-INH complexes was observed. Based on NH₂-terminal sequence analysis of the patients' M_r 96,000 C1-INH, it is concluded that this fragment is generated after cleavage of C1-INH in its active site by one of its target proteases without generating a covalent C1-INH-enzyme complex. In a second patient with a four to five times higher anti-C1-INH antibody titer, the infusion of 500 ml of plasma or of 2,000 U of C1-INH concentrate influenced neither the severity of the patient's angioedema nor the tested parameters, except for an increase in the amount of C1-INH-anti-C1-INH complexes. Analysis of patients' anti-C1-INH antibodies revealed that the antibodies recognize different epitopes within the C1-INH. This suggests that patients with acquired angioedema type II are a heterogeneous group with respect to the C1-INH autoantibodies.

Introduction

The complement component C1-inhibitor (C1-INH)¹ controls the spontaneous autoactivation of the first complement component (C1) as well as activated C1 (1). In humans a functional deficiency of C1-INH is associated with complement activation and recurrent angioedema (2, 3). The syndrome of hereditary angioedema (HAE) is the most common form of this disease and is related either to a decreased synthesis of apparently normal C1-INH protein (HAE type I) (4–6) or to the presence of a dysfunctional protein (HAE type II) (7–9). A deficiency of C1-INH may also be acquired and is usually

associated with a lymphoproliferative disease or other malignancies (acquired C1-INH deficiency type I, acquired angioedema [AAE] type I) (10–13). Both HAE and AAE are characterized by low levels or absence of C2, C4, and functionally active C1-INH (6, 10, 14). In contrast to the hereditary form, patients with AAE show a marked decrease of C1 levels, a normal or slightly increased C1-INH synthesis, and an onset of symptoms in middle age (10, 13). The deficiency of C1-INH in these patients has been suggested to result from an accelerated catabolism of C1-INH due to increased activation of C1 (13).

Recently, we have reported a new type of acquired C1-INH deficiency caused by autoantibodies against C1-INH (AAE type II) (15). The serum of these patients contained considerable amounts of an IgG1 anti-C1-INH antibodies that interfered with C1-INH function. In addition, the C1-INH found in these patients had a molecular mass of only 96,000 D (normal C1-INH, 105,000 D) and was functionally inactive. It was present in plasma at 60–70% of the normal protein level of C1-INH. This molecule presumably resulted from the degradation of M_r 105,000 C1-INH by activated C1s in plasma. In contrast to AAE type I, no other associated diseases were diagnosed in this new type of AAE. Presently, five patients with AAE type II have been reported, three with IgG and two with IgA autoantibodies to C1-INH (15–18).

Long-term prophylactic drug treatment with antifibrinolytic agents, danazol, or other androgens is often successfully used to prevent the potentially lethal manifestations of angioedema in patients with HAE or AAE type I (6, 14, 19, 20). Acute, life-threatening attacks in these types of angioedema have been successfully managed by replacement therapy with C1-INH concentrate (21–23). However, drug treatment in patients with AAE type II failed to correct the complement abnormalities (15, 24). In addition, one patient was found to be resistant to replacement therapy with purified C1-INH or fresh frozen plasma (15).

In this study we investigated the efficacy of C1-INH replacement therapy in two patients with AAE type II and studied several biochemical parameters in patients' plasma after administration of C1-INH. Some of the factors responsible for the therapy resistance of these patients were determined. In addition, the patients' C1-INH molecules and anti-C1-INH antibodies were characterized by sequence analysis and binding studies, respectively.

Methods

Protein purification. C1-INH and activated C1s were purified as previously described (15, 25). In brief, M_r 105,000 C1-INH or patients' C1-INH was affinity purified from plasma on a MAb 13E1 Sepharose column. This antibody recognized the M_r 96,000 C1-INH fragment. After washing the column, bound C1-INH was eluted with 3 M

This work was presented in part at the XIIth International Complement Workshop, Chamonix, France.

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Received for publication 19 July 1988 and in revised form 23 November 1988.

1. *Abbreviations used in this paper:* AAE, acquired angioedema; C1-INH, C1-inhibitor; HAE, hereditary angioedema.

J. Clin. Invest.

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0021-9738/89/06/1794/06 \$2.00

Volume 83, June 1989, 1794–1799

NaCl/50% ethyleneglycol/PBS and dialyzed against PBS. C1-INH was labeled with ^{125}I as described (15).

C1-INH replacement therapy. The C1-INH concentrate (500 U, C1-Inactivator HS; Behring, Marburg, FRG) was reconstituted with sterile distilled water to a final volume of 10 ml and administered intravenously for a period of 5–10 min. The functional activity of 1 U of C1-INH concentrate corresponded to the C1-INH activity in 1 ml of normal human plasma. The C1-INH preparation was homogenous on SDS-PAGE and contained < 3% of functionally inactive M_r 96,000 C1-INH. Plasma samples were collected in heparinized tubes before infusion and at different time points (Table I) after administration and stored at -80°C .

Hemolytic assays. Determination of CH50 (26) and of complement components C1, C2, and C4 (27) was performed as previously described.

ELISA. The details of the experimental conditions (buffers, temperature, volumes, and incubation times) have been previously described (15, 25). The protein concentration of C1-INH and its functional activity were determined as described (25).

Binding of patients' autoantibodies to native C1-INH (M_r 105,000) and to patients' C1-INH (M_r 96,000) was determined on C1-INH-coated microtiter plates according to a previously described method (15). C1-INH bound to the ELISA plate was quantitated by incubation with serially diluted rabbit anti-C1-INH antibodies (Dakopatts, Copenhagen, Denmark), followed by a 1:1,000 dilution of peroxidase-conjugated goat anti-rabbit IgG antibodies (Bio-Rad Laboratories, Munich, FRG). For the detection of C1-INH-anti-C1-INH antibody complexes in plasma, microtiter plates were coated with a 1:200 dilution of polyclonal anti-C1-INH antibodies (Dakopatts) and incubated with plasma, and bound complexes were detected by a 1:200 dilution of peroxidase-labeled rabbit antibody to human IgG (Dako). The maximal amount of C1-INH-anti-C1-INH complexes attainable in patients' plasma was determined by preincubation of plasma with equal volumes of various concentrations of purified native C1-INH for 30 min. The resulting immune complexes were detected with microtiter plates coated with polyclonal anti-C1-INH antibodies as described above. To determine the binding of patients' anti-C1-INH autoantibodies to C1-INH fixed to mouse monoclonal anti-C1-INH antibody 88G2 (28, 29), microtiter plates were coated with 88G2 (10 $\mu\text{g}/\text{ml}$) and incubated with C1-INH (20 $\mu\text{g}/\text{ml}$). Serially diluted plasma

was added and human IgG antibodies bound to C1-INH were detected as described above.

SDS-PAGE and autoradiography. SDS-PAGE was performed as previously described using 5–20% (wt/vol) gradient gels (15). The gels were dried and exposed to x-ray films (Eastman Kodak Co., Rochester, NY) for 1–3 h.

Degradation of C1-INH in patients' plasma in vitro. The degradation of ^{125}I -C1-INH in patients' plasma was determined by SDS-PAGE after incubating 10 μl of ^{125}I -C1-INH (4×10^5 cpm) with 50 μl of plasma for 3 h at 37°C (15). 10 μl of the sample was then analyzed on SDS-PAGE.

Amino acid sequence analysis. Amino-terminal sequence analysis was performed by Edman degradation using 100 μg of the patients' affinity-purified C1-INH and a gas phase sequencer (740A; Applied Biosystems, Foster City, CA) with an on-line 120A PTH analyzer (30).

Results

Replacement therapy with C1-INH in patients with AAE type II

Patient 1. Patient 1 (patient 2 from our previous study; 15) was treated with C1-INH concentrate on two different occasions. In our first study, patient 1 received 500 U of C1-INH concentrate (Table I, day 1, 13.30 h) during an acute attack of angioedema with swelling of the lips, upon which he reacted with more severe edema. Administration of an additional 500 U of concentrate shortly after the first injection (day 1, 14.00 h) interrupted the progression of edema formation, which then resolved within 5 h. Towards the end of this study the patient had a second attack and received another 500 U of C1-INH concentrate (day 4, 8.30 h). In response to the first C1-INH injection, functional C1-INH activity increased up to 60% of normal within 5 min, but dropped to 25% within the next 10 min. After the second infusion, C1-INH activity in plasma increased to 80–85% of normal and remained at this level for the next 2 d. The amount of C1-INH-anti-C1-INH complexes increased 2- to 2.5-fold within 15 min after the first infusion.

Table I. Complement and Anti-C1-INH Autoantibody Levels after Intravenous Administration of C1-INH Concentrate in a Patient with Acquired C1-INH Deficiency Type II

	Day 1					Day 2			Day 3		Day 4				NHP
	↓ 1:30 p.m.	1:35 p.m.	1:45 p.m.	↓ 2:00 p.m.	3:00 p.m.	8:30 a.m.	12:00 p.m.	4:00 p.m.	8:30 a.m.	4:00 p.m.	↓ 8:30 a.m.	9:00 a.m.	9:30 a.m.	12:30 p.m.	
CH50 (U/ml)	0	0	0	2	5	20	23	22	25	23	22	23	22	22	25
C1 (U/ml $\times 10^3$)	1	1	1	2	3	17	21	19	36	38	45	46	45	46	75
C4 (U/ml $\times 10^2$)	1	2	5	7	20	18	15	11	3	1	1	1	1	3	80
C1-INH protein (mg/dl)	12	13	13	13	14	13	13	13	13	12	12	13	12	13	20
C1-INH functional activity (% of normal)	0	60	25	20	60	80	80	80	85	85	80	95	95	90	100
Anti-C1-INH-antibody titer ($\times 10^2$)*	5	6	5	6	7	6	7	6	6	7	7	7	7	7	0
C1-INH-anti-C1-INH complexes titer ($\times 10$)*	8	7	18	20	20	21	20	20	25	26	25	27	28	28	0

500 U of C1-INH concentrate was injected at time points indicated by arrows. CH50, C1, C4, C1-INH protein, functional activity, anti-C1-INH antibodies, and C1-INH-anti-C1-INH complexes were determined in plasma samples collected at the indicated times. NHP, normal human plasma. * Dilution of plasma at which 50% of maximal binding occurs.

Even though no further drastic increase of complexes was observed after the second injection, there was a slow but constant increase in C1-INH-anti-C1-INH complexes until the end of this study. C4 hemolytic activity started to increase after the first injection and reached 25% of normal levels after 1.5 h. It remained stable for ~ 18 h but then returned to preinfusion levels. In contrast to C4, C1 hemolytic activity increased very slowly and reached its maximal activity of ~ 60% of normal at the end of this study. The immunochemically detectable levels of C1-INH protein and of anti-C1-INH autoantibodies remained unchanged during this study.

Since earlier *in vitro* studies showed that ^{125}I -labeled M_r 105,000 C1-INH is rapidly degraded to a M_r 96,000 C1-INH in patients' plasma (15), we tested the collected plasma samples for their ability to cleave intact C1-INH. The results presented in Fig. 1 show that degradation of ^{125}I -C1-INH (track 1) to M_r 96,000 C1-INH was completely abrogated after the first injection of C1-INH concentrate (track 2), but reappeared when C1-INH functional activity decreased (tracks 3 and 4; for comparison see also Table I). Infusion of another 500 U of C1-INH concentrate completely abolished ^{125}I -C1-INH cleavage for ~ 40 h (tracks 5–9). Samples collected before (track 10) and during the onset of a new attack of angioedema (track 11) again showed a similar cleavage of ^{125}I -C1-INH that disappeared after administration of another 500 U of concentrate (tracks 12–14).

During another acute episode of angioedema (second study), the same patient received 1,000 U of C1-INH concentrate at a time. In contrast to the first study where 2×500 U of concentrate were injected with a delay of 30 min, no exacerbation of the edema occurred and the patient's symptoms resolved within 10–15 h. C1-INH functional activity returned to 80% of normal serum levels within 30 min and C4 hemolytic activity peaked 6–7 h after the injection. As in the first study, the amount of C1-INH-anti-C1-INH complexes increased about threefold after the injection, whereas the amount of C1-INH protein and of anti-C1-INH autoantibodies remained unchanged (data not shown).

Patient 2. The second patient (patient 1 in our previous study) had a four to five times higher anti-C1-INH antibody titer than patient 1 (15). In two different severe episodes of laryngeal edema this patient did not respond to infusion of 500 ml of fresh frozen plasma or 2,000 U of C1-INH. Except for an increase (two- and sixfold) in C1-INH-anti-C1-INH antibody complexes immediately after the infusions, all other parameters tested above remained unchanged in samples collected at various time points after therapy with C1-INH (not shown). In addition, no significant changes in edema formation were observed in this patient during the replacement therapy with C1-INH.

Amino acid sequence analysis of patients' C1-INH

While the C1-INH in normal human serum had an M_r of 105,000 in SDS-PAGE, the patients' C1-INH had an M_r of only 96,000 (15). To determine the M_r differences between these two forms of C1-INH, C1-INH from patient plasma or C1-INH from normal human plasma was affinity purified and its NH_2 -terminal amino acid sequence was analyzed by automated Edman degradation. When normal C1-INH (M_r 105,000) was subjected to amino-terminal sequence analysis, a unique amino acid sequence was identified, the sequence being identical to the amino terminus of the C1-INH molecule (data not shown). In contrast, when the patients' C1-INH (M_r 96,000) was sequenced, in each cycle a double amino acid sequence was obtained, corresponding to the amino terminus of intact C1-INH and to the sequence starting at position 445 of the C1-INH sequence (Fig. 2). Quantitation of the phenylthiohydantoin-amino acids showed a 1:1 ratio of the M_r 96,000 fragment of C1-INH (residues 1–444) and of the 34 amino acid peptide (residues 445–478). This suggested that C1-INH is cleaved by one of its target proteases between the Arg-Thr bond (residues 444 and 445) in the active site of C1-INH (Fig. 2).

Since the MAb (13E1) used for the affinity purification of C1-INH is directed against the 96,000-D fragment (residues 1–444), it is of interest that the small carboxyl-terminal fragment of C1-INH (residues 445–478) was copurified on this column. This indicated that this 34 amino acid peptide remains tightly bound to the 96,000-D fragment after the cleavage of C1-INH by one of its target proteases. This is in accordance with previous findings showing that denaturing conditions (1% SDS) are required to separate this small peptide from C1-INH-C1s complexes (31).

Analysis of patients' anti-C1-INH antibodies

During the analysis of patients' anti-C1-INH antibodies we found that the patients' anti-C1-INH antibody titers (Fig. 3 A) did not correlate with the amount of C1-INH-anti-C1-INH complexes detected in plasma (Fig. 3 B). Although patient 2 had an antibody titer approximately four times higher than patient 1, the latter had ~ 8–10 times more C1-INH-anti-C1-INH complexes in plasma. Addition of purified M_r 105,000 C1-INH to plasma increased the amount of complexes *in vitro* ~ 4 and 100 times in patients 1 and 2, respectively. This discrepancy was further analyzed by comparing the binding of anti-C1-INH antibodies to M_r 96,000 C1-INH and to M_r 105,000 C1-INH. Fig. 3 C shows that patient 2 had a four times higher antibody titer than patient 1 when tested with M_r 105,000 C1-INH; whereas, when tested with M_r 96,000 C1-INH, patient 1 had a two to three times higher antibody titer than patient 2. A polyclonal antibody to C1-INH bound to

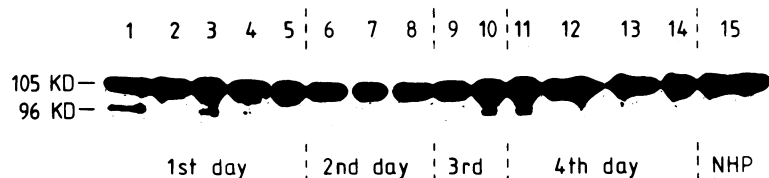


Figure 1. Cleavage of ^{125}I -labeled C1-INH in plasma samples collected before and after the infusion of C1-INH concentrate in patient 1 with acquired angioedema type II. Plasma samples collected during the replacement therapy were incubated with ^{125}I -labeled C1-INH for 3 h at 37°C , separated in 5–20% SDS-PAGE, and exposed to x-ray films. Samples were collected at the following time points: day 1, 1:30 p.m.(1), 1:35 p.m.(2), 1:45 p.m.(3), 2

p.m.(4), and 3 p.m.(5); day 2, 8:30 a.m.(6), 12 p.m.(7), and 4 p.m.(8); day 3, 8:30 a.m.(9) and 4 p.m.(10); and day 4, 8:30 a.m.(11), 9 a.m.(12), 9:30 a.m.(13), and 12:30 p.m.(14). Normal human plasma (NHP) served as a control(15).

normal, although the amount of C1-INH-anti-C1-INH complexes again rose threefold after the injection. Therefore, it is very likely that patients with AAE type II require more C1-INH to abrogate an attack of angioedema than those with other types of angioedema since the autoantibodies have to be neutralized before a sufficient plasma level of functionally active C1-INH can be achieved.

In vitro, the amount of C1-INH required to neutralize the anti-C1-INH antibodies may be calculated by adding purified C1-INH to plasma and assessing the amount of C1-INH-anti-C1-INH complexes. By using an ELISA, the addition of 40 (patient 1) or 400 μ g (patient 2) of purified C1-INH to 1 ml of plasma resulted in a maximum immune complex formation (data not shown), indicating a complete neutralization of anti-C1-INH antibodies. These amounts of C1-INH correspond to the infusion of 600 U (1 U is equivalent to 1 ml of plasma or 0.2 mg of C1-INH) and of 6,000 U of C1-INH concentrate into patient 1 and patient 2, respectively (an assumed average total plasma volume of 3 liters). Therefore, the calculated C1-INH dosages correspond to the evaluated effective dosage in the case of patient 1 and explain the inefficacy of 2,000 U of C1-INH in patient 2.

Throughout our study we did not see any significant changes in the amount of the patients' anti-C1-INH antibodies. However, the slight increase in C1-INH-anti-C1-INH complexes between the days 2 and 4 remains to be explained. After reaching a sufficient plasma level of C1-INH functional activity the response of patient 1 was found to be similar to the one reported for HAE and AAE type I patients (11, 20–23). C4 hemolytic activity transiently increased to 25% of normal levels and C1 titers reached 60% of normal at the end of the study. As previously suggested, this delay in increment in C1 and C4 titers might be due to the synthesis of new protein and/or the decreased catabolism of these proteins (13, 27). Interestingly, the C4 hemolytic activity decreased again after \sim 18 h, although C1-INH functional activity was still 80–85% of normal (Table I). Since even small soluble immune complexes have been found to activate C1 in the presence of diminished levels of C1-INH (32), the drop in C4 titer may be due to the consumption of C4 by activated C1 in the presence of patient's C1-INH-anti-C1-INH complexes.

Amino-terminal amino acid sequence analysis showed that C1-INH in these patients is cleaved between the Arg-Thr bond (residues 444–445) in its active site, presumably by one of its target proteases. In contrast to normal human serum, where such a cleavage results in the formation of irreversible, covalent C1-INH-enzyme complexes (33), no stable complexes were formed in the patients' plasma. Here these complexes seem to dissociate again into the active serine esterase and the modified, inactive C1-INH (M_r 96,000). This dissociation was postulated to be due to the presence of the autoantibodies to C1-INH (34, 35). Therefore, these antibodies may modify C1-INH-target protease interactions as follows:

normal individuals, $E + I \rightleftharpoons E:I \rightarrow E-I^*$;

AAE type II patients, $E + I \rightleftharpoons E:I \rightarrow E-I^* \rightarrow E + I^*$,

where E = enzyme, I = C1-INH, and I^* = modified, inactive C1-INH. According to this scheme, the enzyme is released in an active form and can bind to and inactivate another C1-INH molecule. Therefore, small amounts of active enzyme may

consume large amounts of C1-INH, thus decreasing the half-life of functionally active C1-INH in AAE type II patients.

We have also shown in this study that the patients' anti-C1-INH antibodies recognize different epitopes in the C1-INH molecule (Figs. 3 and 4). This indicates that patients with AAE type II are heterogenous with respect to their anti-C1-INH antibodies. The antibody of patient 2 was found to recognize the 105,000-D C1-INH more than 100 times better than the 96,000-D C1-INH when compared with only a fourfold difference in patient 1 (Fig. 3 C). Therefore, the discrepancy between the titer of the antibody and the amount of C1-INH-anti-C1-INH complexes in the patients' plasma is due to a difference in the recognition of the 96,000-D C1-INH by the patients' antibodies (Fig. 3 A and B).

In regard to C1-INH replacement therapy, the difference in the recognition of M_r 105,000 C1-INH and M_r 96,000 C1-INH may also be of therapeutic relevance. The low affinity of the antibody for the M_r 96,000 C1-INH may indicate that C1-INH injected into patient 2 will not only bind to free autoantibodies, but will also dissociate the existing M_r 96,000 C1-INH-anti-C1-INH complexes to form the more stable M_r 105,000 C1-INH-anti-C1-INH antibody complexes. In contrast, the anti-C1-INH antibody of patient 1 will form more stable complexes with the M_r 96,000 C1-INH and the injected native C1-INH would not dissociate the existing complexes. As a consequence, patient 2 would require more C1-INH concentrate to neutralize the autoantibodies than patient 1. Therefore, these differences in the affinity of the anti-C1-INH autoantibodies for the M_r 105,000 C1-INH and the M_r 96,000 C1-INH could be useful to predict whether or not C1-INH replacement therapy will be successful in a given patient suffering from AAE type II.

In summary, the responses of two patients suffering from AAE type II to replacement therapy with C1-INH were assessed. In comparison to HAE or AAE type I, patients with AAE type II were found to require higher dosages of C1-INH to overcome the neutralizing effect of the anti-C1-INH antibodies present in the plasma and to achieve sufficient plasma levels of functionally active C1-INH. In one patient the efficacy of the treatment was also evidenced by the in vivo synthesis of C4 and C1. However, the therapeutic effect in this patient was only transient, presumably because of the activation of C1 in the presence the C1-INH-anti-C1-INH complexes and the subsequent consumption of C4 and C1-INH. The failure in C1-INH treatment in the second patient most probably was due to the higher anti-C1-INH antibody titer and the lower affinity of the patient's anti-C1-INH autoantibody for the abnormal M_r 96,000 C1-INH.

Acknowledgments

We thank D. Becherer, D. Grossberger, and R. Palacios for critical reading of our manuscript, and M. Zöller-Schütz, G. Lehne, and D. Avila for their excellent technical assistance.

The Basel Institute for Immunology was founded and is entirely supported by F. Hoffman-La Roche Ltd., Co., Basel, Switzerland. J. Alsenz is a recipient of a European Molecular Biology Organization fellowship, ALTF 298-1987.

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