An atypical case of idiopathic nonhistaminergic angioedema with anti-C1-INH antibodies

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Recurrent angioedema without wheals can be hereditary or acquired. The most common form of hereditary angioedema (HAE) is caused by deficiency of C1 esterase inhibitor (C1-INH-HAE), but HAE can also occur with normal plasma levels of C1-INH due to mutations in genes coding for coagulation Factor XII, angiopoietin 1, plasminogen, kininogen 1, myoferlin and heparan sulfate (HS)-glucosamine 3-O-sulfotransferase 6. HAE with unidentified genetic cause is defined as HAE of unknown origin [1].

Acquired angioedema (AAE) includes idiopathic histaminergic AAE (IH-AAE), idiopathic non-histaminergic AAE (InH-AAE), AAE related to angiotensin-converting enzyme inhibitors (ACEI-AAE) and AAE with C1-inhibitor deficiency (C1-INH-AAE) [2].

C1-INH-AAE is a rare disease characterized by cutaneous swellings, edema of the gastrointestinal mucosa and life-threatening laryngeal edema [3]. The onset of symptoms occurs after the fourth decade of life in 90% of patients, and family history of angioedema is absent.

Acquired C1-INH deficiency leads to activation of the complement and contact systems. Complement consumption via the classical complement pathway is associated with reduced C4 in almost all
patients and low C1q in >70% of cases. Contact system activation causes the production of bradykinin and onset of angioedema [4]. Anti-C1-INH antibodies are detected in >70% of cases [5].

In some cases, it is difficult to distinguish C1-INH-AAE from InH-AAE, a condition that comprises nonfamilial, nonhereditary forms in which known causes have been excluded and both C4 and C1-INH are normal [2].

We studied in depth the previously reported case [6], of a 86-year-old woman affected by recurrent angioedema with anti-C1-INH antibodies and normal C1-INH levels. Informed consent for all interventions was obtained from the patient. Her symptoms began when she was aged 66, with recurrent episodes of peripheral edema without wheals (1 episode/month), abdominal attacks and occasional episodes of upper airway edema. She was first treated with antihistamines, corticosteroids and epinephrine without benefit. None of her relatives had ever experienced angioedema. Since 2011 she has been treating attacks with off-label subcutaneous injection of icatibant (an antagonist of bradykinin B2 receptors) 30 mg on demand, with improvement after 20-30 minutes and resolution after 8-20 hours. No adverse events were reported.

Since her history was typical of bradykinin-mediated angioedema, we measured levels of C1-INH, C4, and C1q using standardized assays (par.1, Supplementary Material), all of which were normal and remained within normal ranges during 10 years of follow-up. Nevertheless, we suspected C1-INH-AAE. Indeed in some patients at disease onset, consumption of complement components is evident only during angioedema attacks. We investigated anti-C1-INH antibodies, using plasma derived C1-INH to coat an ELISA plate (par.2, Supplementary Material) and we detected anti-C1-INH IgG (Supplementary fig.1). In order to evaluate whether C1-INH polysaccharides could be a target for the autoantibodies, we used recombinant C1-INH, that has a different glycosylation pattern compared to plasma derived C1-INH [7], and IgG of the patient targeted recombinant C1-INH as well
Anti-C1q antibodies were negative. Total serum IgG levels were within normal limits. We ruled out underlying autoimmune and lymphoproliferative diseases and monoclonal gammopathy of undetermined significance.

In order to rule out a diagnosis of hereditary angioedema, genes implicated in the onset of the disease were evaluated [8]: SERPING1 (NM_00006.2), ANGPT1 (NM_001146.5), PLG (NM_000301.4), MYOF (NM_013451.4), KNG1 (NM_001102416.3) and F12 (NM_000505.3). No mutation was found.

Functional C1-INH in patient’s plasma was then evaluated using ELISA based on inhibition of complement C1s, kallikrein, or factor XIIa [9] (par.5, Supplementary Material). Our results showed that the level of complexed C1-INH of the patient was negligible compared to normal controls, indeed there was no inhibitory activity towards all tested proteases (Fig.1).

We evaluated cleaved high molecular weight kininogen (cHK), an indirect measure of bradykinin, (par.6, Supplementary Material). The plasma of the patient was characterized by a high value of cHK: 72% versus 33% in 11 healthy subjects (Supplementary fig.3) [10].

Total IgG from patient’s serum was purified (par.7, Supplementary Material) and neutralizing activity towards normal C1-INH was assessed using a chromogenic assay. Complexes between patient’s IgG and C1-INH were allowed to form before mixing with kallikrein or C1s. No effect towards C1-INH inhibition on kallikrein or C1s was observed (Supplementary fig.4). There was no difference in all tested experimental conditions i.e. different amounts of IgG used, or different preparations of complexes, including use of purified autoantibody of the patient isolated from patient IgG passed over a column of purified C1-INH as ligand (data not shown).

The neutralizing capacity of anti-C1-INH antibodies of the patient was also evaluated by means of a specifically designed ELISA (par.10, Supplementary Material). The plate was coated with purified
C1-INH. Quality Controls (QC) and test samples were added. Wells were incubated with either biotinylated C1s, kallikrein or FXIIa, afterwards streptavidin-HRP was allowed to bind the biotinylated ligands (Supplementary fig. 5a-c). The index patients showed a neutralizing capacity comparable to control QC2 for C1s and kallikrein (Supplementary fig. 5a,b), but not for FXIIa (Supplementary fig.5c). Neutralizing capacity in index patient was weaker than in the positive control plasma.

We tested the capacity of the autoantibodies to interfere with the formation of a stable covalent complex between C1-INH and its target proteases. In the first protocol we used, proteases were added exogenously to the patient’s plasma and their complexes with endogenous C1-INH were tested: no complexes were found (Fig. 1). This could be due to the presence of 1) a non-functional C1-INH or 2) a neutralizing antibody against C1-INH, reacting with either C1-INH or with the target proteases.

In the second protocol, the patient’s plasma had a neutralizing effect on the formation of complexes with exogenous C1s and kallikrein, but not activated factor XII (Supplementary fig. 5a-c). This could be due to the presence of 1) an excess of endogenous activated proteases interacting with exogenous C1-INH and preventing exogenous proteases to form complexes (in this case endogenous C1-INH has to be non-functional) or 2) free neutralizing antibodies against C1-INH.

Given the fact that in the chromogenic functional assay the activity of endogenous C1-INH versus exogenous C1s appears in the normal range, the hypothesis of a non-functional C1-INH should be discarded. Moreover, mutations of C1-INH were ruled out.

A proposed hypothesis is that of a neutralizing antibody against C1-INH whose action would make the complex between C1-INH and the protease less stable. In this way the protease is initially inhibited but the short half-life of the complex would finally allow the release of an active protease.
The reaction conditions of the standard chromogenic assay are not suitable to appreciate this phenomenon since longer pre-incubation times would be necessary.

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**Conflicts of interest**

The authors have not conflict of interest to report.
References


Figure.

Functional ELISA based on inhibition of plasma kallikrein (PKa), factor XIIa (FXIIa), and complement C1s (C1s). Results (mean with SD) are expressed as percentage of controls.