Activation of the ficolin-lectin pathway during attacks of hereditary angioedema

Dorottya Csuka, PhD,[a,b] Lea Munthe-Fog, PhD,[b] Estrid Hein, MSc,[b] Zsuzsanna Zotter, MD,[a] Zoltán Prohászka, MD, PhD, DSc,[a] Henriette Farkas, MD, PhD, DSc,[a] Lilian Varga, PhD,[a]* and Peter Garred, MD, PhD, DSc[b]* *Budapest, Hungary, and Copenhagen, Denmark*

Background: The activation of plasma enzyme systems is insufficiently controlled in hereditary angioedema due to the deficiency of C1-inhibitor (C1-INH) (HAE-C1-INH). Recently, it was suggested that the ficolin-lectin pathway (ficolin-LP) might play a more dominant role than the mannose-binding lectin-lectin pathway in the pathomechanism of HAE-C1-INH.
Objective: Because the role of the ficolin-LP during edematous attacks is still enigmatic, we analyzed its activity during such episodes.
Methods: Thirty-five patients with HAE-C1-INH, who have experienced severe attacks on 106 occasions, were enrolled. We analyzed blood samples drawn during attacks, and obtained 35 samples from the same patients during symptom-free periods. The serum levels of ficolin-2, ficolin-3, MASP-2, ficolin-3/MASP-2 complex, C1-INH, and C4, as well as the extent of ficolin-3–mediated terminal complement complex (FCN3-TCC) deposition, were measured using ELISA-based methods.
Results: Levels of MASP-2 and of the ficolin-3/MASP-2 complex were elevated ($P < .0001$ and .033, respectively), whereas that of FCN3-TCC was lower ($P < .0001$) during attacks than during the symptom-free period. During symptom-free periods, FCN3-TCC deposition was significantly related to concentrations of ficolin-3 ($R = 0.2778$; $P = .0022$), antigenic C1-INH ($R = 0.3152$; $P = .0006$), and C4 ($R = 0.5307$; $P < .0001$). Both ficolin-3 and MASP-2 levels correlated inversely with the time from the onset of the attack until blood sampling.
Conclusions: There is a marked heterogeneity of the pathomechanism and development of hereditary angioedema attacks in different patients. Our results suggest that the activation of the ficolin-LP may deplete the innately low level of C1-INH and thus, it may contribute to the uncontrolled activation of plasma cascade systems, and thereby to edema formation. (J Allergy Clin Immunol 2014;134:1388-93.)

Key words: *Hereditary angioedema, C1-inhibitor, ficolins, MASPs, lectin pathway, edematous attack*

In humans, soluble pattern recognition molecules (such as mannose-binding lectin [MBL], ficolin-1, ficolin-2, ficolin-3, and collectin-11), acting in concert with MBL-associated serine proteases (MASP-1 and MASP-2), activate the lectin pathway of the complement system.[1,2] According to preliminary studies, MASP-3 as well as the nonenzymatic proteins MAP-1 and sMAP are also involved in the regulation of this activation.[3-5] The binding of MBL/ficolins to the carbohydrate molecules (mannose, *N*-acetyl-glucosamine, fucose) present on the surface of bacteria, viruses, and other microorganisms, as well as of dead host cells, activates MASP-2. The latter cleaves the complement components C4 and C2, leading to the formation of the terminal complement complex (TCC).[6-8] Both the C1-inhibitor (C1-INH) and alpha-2-macroglobulin play a dominant role in the regulation of lectin pathway activation because they form complexes with MASP-1 and MASP-2[9-11] although they are not able to bind to MASP-3.[12] Considering the prominent role of C1-INH in the regulation of lectin pathway activation, we have studied previously whether the deficiency of C1-INH has a role in the activation of the MBL-lectin pathway or of the ficolin-lectin pathway in hereditary angioedema due to C1-inhibitor deficiency (HAE-C1-INH). Furthermore, we explored its possible effect on the pathomechanism of this disorder. Previous studies conducted in symptom-free patients showed that the levels of antigenic and of functional C1-INH, C4, C2, and MASP-2, as well as the total activity of the MBL-lectin pathway, are suppressed in this disorder.[13] However, the MBL level was not related to any of the clinical indicators, and even MBL–MASP-2 activity correlated only with the quantity of the plasma-derived C1-INH (pdC1-INH) concentrate administered.[14] However, our previous observation implies that the ficolin-mediated activation of the lectin pathway in HAE-C1-INH might be important. In particular, ficolin-2, ficolin-3, MASP-3, and MAP-1 levels were closely correlated with clinical indicators of the severity of HAE-C1-INH (ie, annual attack number, the number of attacks occurring in different locations, and the quantity of pdC1-INH concentrate administered). Our findings suggested that the severity of the disease is inversely related to ficolin levels. That is, lower ficolin-2 and

From [a]the 3rd Department of Internal Medicine, Semmelweis University, Budapest; and [b]the Laboratory of Molecular Medicine, Faculty of Health Sciences, Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen.
*These authors contributed equally to this work.
This research was realized in the frames of TÁMOP 4.2.4. A/1-11-1-2012-0001 "National Excellence Program – Elaborating and operating an inland student and researcher personal support system" (grant no. A1-EPFK-14-0092). The project was subsidized by the European Union and cofinanced by the European Social Fund. The equipment and the infrastructure of the research was supported by the Országos Tudományos Kutatási Alapprogramok (OTKA) 100886 (HF) grant, the Novo Nordisk Research Foundation, the Svend Andersen Foundation, the Danish Medical Research Council, and the Capital Region of Denmark.
Disclosure of potential conflict of interest: D. Csuka has received travel support from Viropharma, CSL Behring, and Shire Human Genetic Therapies, Inc. H. Farkas has received research support from the Hungarian Scientific Research Fund and has consultant arrangements with and has received travel support from Shire Human Genetic Therapies, Inc, Pharming, Viropharma, and CSL Behring. L. Varga has received travel support from CSL Behring and Shire Human Genetic Therapies, Inc. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication September 18, 2013; revised April 26, 2014; accepted for publication May 28, 2014.
Available online July 16, 2014.
Corresponding author: Dorottya Csuka, PhD, Faculty of Medicine, 3rd Department of Internal Medicine, Semmelweis University, H-1125 Budapest, Kútvölgyi út 4, Hungary. E-mail: csuka.dorottya@med.semmelweis-univ.hu.
0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.05.030

Abbreviations used

C1-INH: C1-inhibitor
FCN3-TCC: Ficolin-3–mediated terminal complement complex
HAE-C1-INH: Hereditary angioedema due to the deficiency of C1-inhibitor
LMM: Linear mixed model
MBL: Mannose-binding lectin
pdC1-INH: Plasma-derived C1-INH
TCC: Terminal complement complex

ficolin-3 levels are typical of patients with more severe disease, and this implies the consumption of these components during the activation process.[14] Because of the deficiency of C1-INH, the C1-complex, as well as MASP-1 and MASP-2, may undergo uncontrolled activation, which leads to the increased cleavage of C4.[13,15,16] Both MASP-1 and MASP-2 may be activated and their levels may be elevated during episodes of stress and/or in response to infections The latter may trigger edematous attacks, at least in a proportion of patients with HAE-C1-INH.[16-20] It seems reasonable that excessive activation of the lectin pathway—initiated by the ficolins, and leading to C1-INH consumption by MASP-1 and MASP-2—might throw the regulation of C1-INH off-balance, and thereby lead to edematous attacks via the consumption of C1-INH.

To analyze this issue, our aim was to study the levels of proteins of the ficolin-lectin pathway and its activation in the same patients with HAE-C1-INH, both during symptom-free periods and during oedematous attacks. We also investigated whether the ficolin-lectin pathway undergoes activation during edematous attacks, and studied possible changes in ficolin-lectin pathway parameters that characterize attacks occurring in different locations.

METHODS

Patients with HAE-C1-INH

Thirty-five patients with HAE-C1-INH (12 men and 23 women; median age, 38 years; 25th-75th percentiles, 22-50 years), 29 with HAE-C1-INH type I and 6 with HAE-C1-INH type II, were enrolled in our study. In each patient, HAE-C1-INH was diagnosed according to the accepted clinical and laboratory criteria (positive family history, clinical symptoms of angioedema, low functional C1-INH level, low C4 level, normal C1q).[16] Human plasma–derived C1-INH concentrate (Berinert; CSL Behring, Marburg, Germany) was used as an acute treatment for edematous attacks, or as short-term prophylaxis. All patients received pdC1-INH concentrate in every instance to relieve the most severe forms of edematous episodes (ie, upper airway, abdominal, facial, genital, and severe limb edema, predominantly).

The "symptom-free samples" were collected during the annual control visits. Furthermore, 106 samples in total were collected from the patients during edematous attacks, before administering the pdC1-INH concentrate (at least 1, but no more than 17 "during-attack samples" were obtained per patient). The severity and the location of edematous attacks, as well as the time from the onset until blood sampling, were recorded in the Hungarian HAE Registry.

Submucosal versus subcutaneous attacks

Submucosal edema in the upper airways may progress to involve the larynx within hours and thus cause suffocation. In the gut, the edema of the intestinal wall may mimic the clinical symptoms of an acute abdominal "catastrophe," such as nausea, vomiting, colicky pain, and watery diarrhea, after the attack. During the episode, abdominal ultrasound depicts free peritoneal fluid and in a large proportion of cases, it also confirms edema of the intestinal wall.

Subcutaneous edema most commonly affects the extremities, face, eyelids, neck, torso, gluteal region, and external genitals. The ensuing swelling is painless and it is not accompanied by erythema and pruritus because the edema involves the deeper cutaneous layers containing fewer mastocytes and sensory nerve endings. On the face, subcutaneous edema may extend to involve the mucosa of the upper airways. On the neck, it can cause complications through compression, whereas the subcutaneous swelling of the chest may be accompanied by pericardial or pleural effusion.

Healthy controls

The control group consisted of 54 healthy adults (21 men and 33 women; median age, 33 years; 25th-75th percentiles, 21-58 years). All the subjects had been referred for routine medical check-up, and they had volunteered for the study by giving informed consent. The healthy controls did not have any known disease, or receive medicinal products at the time of blood sampling. C1-INH deficiency was excluded by complement testing in all healthy subjects. Patients with HAE-C1-INH and controls were not statistically different as regards age and sex distribution.

Blood sampling

Serum and EDTA-plasma samples obtained from patients with HAE-C1-INH both during symptom-free periods and during attacks were stored at −70°C until processing. Peripheral blood samples were also drawn from healthy subjects, as prescribed by the study protocol. The latter was approved by the Institutional Review Board of Semmelweis University of Budapest, and informed consent was obtained in accordance with the Declaration of Helsinki.

Measurement of the lectin pathway parameters

The levels of ficolin-2,[21] and ficolin-3,[22] as well as of the ficolin-3/MASP-2 complex[23] were determined by using standard sandwich ELISA techniques, using mAbs specific for each molecule. Biotinylated antibodies were added to the second layer, and streptavidin/horseradish peroxidase complexes were used for detection. All samples were tested in duplicate against a standard serum pool with known content of each analyte.

Ficolin-3–mediated terminal complement complex (FCN3-TCC) deposition was measured as described previously by Hein et al.[24] In brief, acetylated BSA was immobilized in Maxisorb ELISA plates and used as a ficolin-3 ligand. To block any interference from the classical pathway or the alternative pathway,[25] full serum samples were preincubated with sodium polyanethole sulfonate. Serum samples were diluted 1:25 in barbital buffer containing 0.05% Tween-20 and incubated on the plate for 45 minutes at 37°C. Thereafter, mouse-anti-human TCC was applied for 2 hours at room temperature and then, rabbit-anti-mouse-horseradish peroxidase was added to the wells as secondary antibody, for 1 hour at 37°C. Finally, the plates were developed using ortho-phenylenediamine substrate and the OD was determined at 490/630 nm.

Commercial kits were used to quantify the levels of MASP-2 in EDTA-plasma samples. We used a solid-phase ELISA based on the sandwich principle (Hycult Biotech, Inc, Uden, The Netherlands), described by Møller-Kristensen et al.[26] We measured the functional activity of C1-INH in serum samples (Quidel, San Diego, Calif) according to the manufacturer's instructions.

To measure the concentration of C4, radial immunodiffusion was performed using polyclonal rabbit anti-human C4c (Dako, Glostrup, Denmark) and human serum protein calibrator (Dako).

Statistical analysis

Statistical calculations were performed with Prism for Windows (version 5.02; GraphPad Software, Inc, San Diego, Calif; www.graphpad.com) and SPSS (version 13.0; SPSS, Inc, Chicago, Ill). The Mann-Whitney *U* test was used to compare 2 independent groups, whereas the Wilcoxon test was chosen to compare the symptom-free and during-attack results of the same