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Diminished capacity of opsonization and immune complex solubilization, and detection of anti-C1q antibodies in sera from patients with hereditary angioedema

Daisuke Honda [a], Isao Ohsawa [a, b, *], Nobuyuki Sato [a], Hiroyuki Inoshita [a], Satoshi Mano [a], Yasuhiko Tomino [a, c], Yusuke Suzuki [a]

[a] Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan
[b] Nephrology Unit, Saiyu Soka Hospital, Saitama, Japan
[c] Medical Corporation **Showakai**, Tokyo, Japan

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Abbreviations:
C1-INH, C1 esterase inhibitor;
CR, Complement receptor; HAE, Hereditary angioedema; IC, Immune complex;
NHPS, Normal human pooled sera;
PBS, Phosphate buffered saline;
PI, Propidium iodide; PMA, Phorbol-12-myristate-13-acetate; PO, Peroxidase;
SD, Standard deviation; SLE, Systemic lupus erythematosus;
TMB, Tetramethylbenzidine;
HUVS, Hypocomplementemic urticarial vasculitis syndrome

ABSTRACT

Background: Hereditary angioedema (HAE) is an autosomal dominant disease caused by deficiency of C1 esterase inhibitor. Symptoms of HAE include edema, which can potentially cause suffocation. Some patients with HAE exhibit immunological abnormalities, which could prevent an accurate diagnosis. Low levels of complement components are characteristic of HAE and in other settings are thought to reduce elimination of apoptotic cells and immune complex (IC). Thus, we aimed to experimentally clarify the mechanism of immunological abnormalities using sera from HAE patients.
Methods: Serum samples from 18 patients with HAE were collected when free from angioedema attack and compared with normal human pooled sera (NHPS) from 20 healthy volunteers. Opsonization was measured as the rate of phagocytosis of apoptotic Jurkat cells by macrophages differentiated from THP-1 cells incubated with serum. IC solubilization in serum was analyzed by quantifying peroxidase released from a synthetic IC composed of peroxidase and anti-peroxidase antibodies. Anti-C1q antibody levels were detected using an enzyme-linked immunosorbent assay.
Results: Serological immunological abnormalities were detected in 12 patients. Opsonization in serum samples from each patient with HAE was lower than that in NHPS (~20% versus 70%, respectively). The rate of IC solubilization was lower in serum from HAE patients than NHPS. Some patients had high serum anti-C1q antibody levels with increased serum IC levels.
Conclusions: Sera from patients with HAE exhibit anti-C1q antibodies, with a lower capacity for opsonization and IC solubilization. This may be associated with immunological abnormalities and should be investigated further to facilitate accurate diagnosis of HAE.

Introduction

Hereditary angioedema (HAE) is a rare autosomal dominant disease caused by mutations in the C1 esterase inhibitor (C1-INH) gene. These mutations cause deficiencies in the expression (HAE type I) or functional activity (HAE type II) of C1-INH, which can lead to continuous and uncontrolled activation of the complement, contact, blood coagulation, and kallikrein-kinin cascades.[1,2] Consequently, the release of bradykinin, a vasoactive peptide in the kallikrein-kinin system, induces acute episodes of localized subcutaneous or submucosal angioedema in the extremities, limbs, torso, face, gastrointestinal tract, genitals, larynx, and trachea.[1,2] These episodes can be triggered by stress, minor trauma, drugs, surgical procedures and other unknown reasons.[1] A third type of HAE has also recently been reported, where patients exhibit normal C1-INH levels but have mutations in the gene for clotting factor XII.[3,4]

* Corresponding author. Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, 2-1-1 Hongo, Bunkyo-Ku, Tokyo 113-8421, Japan.
E-mail address: iohsawa@juntendo.ac.jp (I. Ohsawa).
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Although HAE is a rare disease, estimated to affect around 1 in 50,000 individuals, acute attacks of edema are life-threatening and can cause suffocation if they develop in the upper airways.[5] Thus, it is essential that HAE is accurately and rapidly diagnosed and appropriately treated. Plasma-derived human C1-INH was approved for treatment of HAE attacks in Japan in 1990 and has been first-line therapy in Europe and other countries for several decades.[6] Despite this, awareness of HAE is low and in Japan the mean time to diagnosis from first symptoms was reported to be 13.8 years.[7] According to the World Allergy Organization guideline for the management of HAE, patients with suspected HAE should be screened for low serum levels of C4, and then diagnosed by low functional and antigenic levels of C1-INH.[8] However, since it has been reported that a proportion of HAE patients show immunological abnormalities,[9–12] these abnormal findings could mimic other disorders and further delay or prevent an accurate diagnosis of HAE.

Clinical laboratory findings from patients with HAE have revealed chronic activation and consumption of the complement system.[2,13] In patients with HAE, low levels of expression or impaired functional activity of C1-INH causes a gradual reduction in serum levels of complement proteins including C4 and C2.[2,13] It is well established that the complement system plays an important role in removal of apoptotic cells and immune complexes (IC), as well as IC solubilization.[14,15] In particular, C1, C3 and C4, which are early complement components of the classical pathway, are required as opsonins for complement-mediated phagocytosis, and C1, C4 and C2 are essential for IC solubilization and clearance.[16,17] Low levels of complement components may reduce the elimination rate of apoptotic cells and IC, which may potentially become sources of autoantigens that could lead to autoimmunity and other immunological abnormalities.[18,19] Genetic deficiencies in complement components such as C1q or C4 have been associated with autoimmune diseases such as systemic lupus erythematosus (SLE).[20,21] In fact, the prevalence of definitive autoimmune disorders in patients with HAE has been reported to range from 0.4 to 12%.[9]

The aim of the present study was to determine the cause of immunological abnormalities in patients with HAE. We hypothesized that low levels of early complement components, which are a characteristic of HAE, may affect opsonization and IC solubilization.

Methods

Patients and blood samples

The study enrolled all patients who had a confirmed genetic diagnosis of HAE at Juntendo University Hospital between November 2013 and October 2014. Study procedures were performed in accordance with the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of Juntendo University (No. 25-325). Written informed consent was obtained from all participants prior to inclusion.

Serum samples were collected from patients with HAE when they were not experiencing angioedema attack. In parallel, serum samples were obtained from 20 healthy volunteers without autoimmune disease or clinical symptoms of angioedema, as described previously[16]; these were used as a control and referred to as normal human pooled sera (NHPS). Serum samples were stored at −80 °C in 500-μL aliquots and each aliquot was only used once.

Laboratory data

Using standard laboratory assays, the following clinical parameters were analyzed in patients with HAE and healthy volunteers: C1-INH activity, serum C1-INH levels, serum C4 and C1q levels, the quantity of IC (C1q-binding assay), and the presence of antinuclear antibodies and/or cryoprecipitate (protein, mostly immune complexes, that become insoluble at reduced temperature and are associated with various diseases) in serum.

Evaluation of opsonization capacity

A human monocytic leukemia THP-1 cell line (Public Health England, London, UK) was cultured in RPMI 1640 medium containing 2 mM L-glutamine (Sigma–Aldrich, St. Louis, USA), supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, USA). Floating, non-dendritic THP-1 cells were differentiated into adherent macrophages by the addition of 100 nM phorbol-12-myristate-13-acetate (PMA) (Abcam, Cambridge, UK), as described previously.[22,23]

Induction of apoptosis in human T-cell lymphoma Jurkat cells (Public Health England) was performed as previously described,[21,24] with minor modifications as follows. Jurkat cells were suspended in RPMI 1640 medium containing 1% penicillin/streptomycin and irradiated with ultraviolet light (8 W, 170 mJ/cm^2) using a FUNA-UV-LINKER (Funakoshi, Tokyo, Japan). Cells were incubated for 4 h or 24 h at 37 °C in 5% CO_2. For detection of apoptosis they were stained using an Annexin V-FITC kit (Beckman Coulter, Brea, CA, USA), in which Annexin V binds to phosphatidyl serine located on the outer cell membrane only during early apoptosis, and propidium iodide (PI) is used to visualize DNA content and integrity. Cells were sorted via flow cytometry using a FACScan system (Salk Institute, San Diego, CA, USA).

Phagocytosis assays were conducted as previously described,[23,24] with minor modifications as follows. Serum samples from patients with HAE were analyzed, using NHPS as a control. Apoptotic cells (1×10^6 cells/mL) and macrophages (1×10^5 cells/mL) were incubated with 30% serum for 1 h at 37 °C in a 25 × 75-mm chamber slide (Thermo Fisher Scientific). After 1 h incubation, the chamber slides were gently washed with phosphate buffered saline (PBS) to remove non-bound apoptotic cells. The chamber slides were air-dried, fixed with methanol for 10 min, and stained with hematoxylin-eosin. The phagocytosis index, as a measure of the opsonization capacity, was defined as the number of macrophages binding to apoptotic cells or undergoing phagocytosis per 100 macrophages. Slides were visualized and scored at ×400 magnification using standard light microscopy. Each experiment was performed in duplicate and the phagocytosis index was expressed as the mean average.

To detect the deposition of complement factors on the cell surface, irradiated apoptotic cells were incubated with 30% NHPS or 30% sera from patients with HAE for 1 h at 37 °C. Each aliquot was incubated with anti-C1q mouse antibody (1:10 dilution; Abcam), anti-C4d rabbit antibody (1:20 dilution; Abcam) or anti-iC3b mouse antibody (1:400 dilution; Genway, San Diego, CA, USA) for 1 h at room temperature after the addition of a human Fc receptor blocking reagent, according to the manufacturer's protocol (MBL, Nagoya, Japan). After several gentle washes with PBS, the cell aliquots were incubated at room temperature for 1 h with secondary goat anti-mouse antibody or donkey anti-rabbit antibody (both 1:100 dilution; Thermo Fisher Scientific). After several further washes, apoptotic cells with complement factors on the cell surface were detected by flow cytometry.

Macrophages and monocytes have been reported to express cell surface complement receptors (CRs), including CR1 (CD35), CR3 (CD11b/CD18) and CR4 (CD11c/CD18).[25] To elucidate the role of cell surface complement receptors in phagocytosis, CR blockade was attempted by incubating macrophages with an anti-CR1 antibody (10 μg/mL), an anti-CR3 antibody (10 μg/mL) and an anti-CR4 antibody (5 μg/mL; all from Abcam), as described previously.[26]