Complement Activation in Sudden Deafness

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Objective: To test whether complement activation is associated with sudden deafness.

Design: Plasma samples obtained from patients in the acute phase of sudden deafness were analyzed for complement activation measured by C3bc levels and terminal complement complex. Comparisons were made with plasma samples from healthy controls.

Patients: Twenty-five adult patients with unilateral sudden deafness. The criteria for inclusion were symptoms of hearing loss for not longer than 14 days and a hearing loss of 35 dB or more measured at entry.

Results: Levels of C3bc were higher in patients compared with controls (P<.001). There were no differences in the formation of terminal complement complex in patients and controls.

Conclusions: The elevated levels of C3bc in patients with sudden deafness indicate an activation of the first part of the complement cascade and therefore suspected inflammatory causes. Measurements of C3bc levels might identify patients with sudden deafness who would benefit from treatment with anti-inflammatory drugs.


Sudden deafness (SD) is defined as a sensorineural hearing loss of varying degrees that develops instantaneously or within hours. Its severity varies from mild to total hearing loss. Patients' recovery rates vary from 90% in mild cases to 5% in profound cases, depending on several factors, such as severity of the initial hearing loss, associated vertigo, the patient's age, and hearing ability in the contralateral ear.

In most cases of SD, the cause remains unknown. Several causes have been considered, such as vascular disorders, neoplasms, trauma, membrane rupture of the oval or round window, and inflammation due to viral, bacterial, or autoimmune disease.

Our knowledge of the complement system has expanded considerably in recent decades. This cascade system consists of approximately 30 proteins that act synergistically in a particular sequence with the main function of inducing an inflammatory reaction. The complement system is an important barrier against infections, but due to its inflammatory effect, it can also contribute to the destruction of tissue in a variety of autoimmune and inflammatory diseases not necessarily caused by microbes.

Complement can be activated through either the classic or the alternative pathway, but by whichever route, factor C3, the central component in the first part of the cascade, is activated. Complement factors C5, C6, C7, C8, and C9 constitute the terminal pathway, and their activation leads to an aggregation called the terminal complement complex (TCC). Activated C3 can be identified by measuring its activation product (C3bc), which indicates activation of the first part of the cascade, whereas TCC indicates activation of the terminal pathway.

Since 1979, it has been suspected that there might be an autoimmune cause in some cases of sudden deafness. Various authors have tested several nonspecific antibodies against different cellular elements. Prior to this study, we examined patients with SD for laboratory parameters that might indicate an autoimmune pathogenesis and found indications of complement activation.

The aim of the present study was to investigate the occurrence of complement activation in patients with SD, measured by levels of C3bc and TCC in plasma.
PATIENTS, MATERIALS, AND METHODS

Adult patients (aged ≥18 years) with unilateral SD occurring within 14 days of their first visit to the clinic were included in this prospective study; all but 2 patients attended within 7 days. For each patient, a history of indications of autoimmune disease was carefully recorded. The criterion for SD was a hearing loss of a pure tone average (0.5-2.0 kHz) of 35 dB or more, compared with results from previous audiograms or normal hearing ability. Hearing loss was measured as a pure tone threshold in the range of 0.125 to 8.0 kHz. The study group consisted of 25 patients (17 men and 8 women), all of whom fulfilled the inclusion criteria. The mean age was 56.3 years (age range, 22-81 years).

Plasma samples from the patients were collected within 24 hours of arrival at the hospital. Plasma samples from an unmatched group of 24 healthy control subjects were collected during the same period. All samples were obtained using EDTA in blood, prepared at 4°C, and stored at −70°C according to guidelines for samples to be analyzed for complement activation.6 The samples from the case patients were analyzed in a single batch after the study was concluded. All samples from both patients and controls were labeled with codes to render the testing blind to the laboratory staff.

LABORATORY ANALYSES

Complement activation was measured using the new generation of quantitative enzyme immunoassays based on monoclonal antibodies that are highly specific for neoepitopes exposed in the activation products but hidden in the native components.7 These immunoassays were used because they enable direct and reliable detection of complement activation without in vitro activation, in contrast to many previous assays that were hampered with procedures required to separate activation products from native components.

C3 activation products (C3bc) were measured using the monoclonal bH6 antibody reacting with a neoepitope expressed in C3b, iC3b, and C3c, but not in native C3.8 The high degree of specificity for this antibody is described by Garred et al.9 Microtiter plates (Immunoplate Maxi Sorp, NUNC, Copenhagen, Denmark) were coated with ascitic fluid of a monoclonal antibody bH6 (produced in our laboratory) and diluted in phosphate-buffered saline (PBS) overnight at 4°C (100 µL in each well). The plates were washed 3 times (200-µL washing volume) in an automatic washer (Dynawasher, Dynatech, Denkendorf, Germany). Plasma samples were diluted 1:150 in PBS containing 0.1% polysorbate 20 (Tweez 20) and 10-mmol/L EDTA (PBS-T) and added in triplicate (50 µL in each well). The standard was zymosan-activated serum (10 mg of zymosan per milliliter of serum incubated for 1 hour at 37°C and centrifuged for 45 minutes at 10,000g), which contained 1000 units of C3bc per milliliter. The standard curve was constructed by a 2-fold dilution ranging from 1:800 to 1:51200 in PBS-T (50 µL in each well). The plates were incubated for 1 hour at 4°C and then washed. Detection antibody (rabbit polyclonal anti-C3c [Behringwerke AG, Marburg, Germany]) was diluted 1:10000 in PBS-T and added to the wells (100 µL in each), and the plates were incubated for 1 hour at 37°C. After washing, the conjugate (rabbit Ig [American, Buckinghamshire, England]) was diluted 1:1000 in PBS-T, added to the wells (100 µL in each), and the plates were incubated for another hour at 37°C. After washing, the substrate was added (100 µL in each well), which contained 0.3-mmol/L 2,2-azino-di(3-ethyl)benzthiazoline sulphonate (ABTS) diluted in a 0.15-mmol/L acetate buffer (pH, 4.0) to which hydrogen peroxide was added to a final concentration of 2.4 × 10−3% immediately before use. After 30 minutes, the plates were read in a Dynatech MR7000 reader (Dynatech) at 410 nm, using 490 nm as a reference filter.

The TCC was quantified using the monoclonal aE11 antibody specific for a C9 neoepitope expressed in C9 when incorporated into TCC, but not expressed in native C9.10 The high degree of specificity for this antibody has been described previously.11 The details of our enzyme immunoassay were exactly as described previously for C3 activation products with respect to incubation times, volumes, buffers, substrates, and readings.8 Our standard was also the same, defined to contain 1000 units of TCC per milliliter. The plates were coated with ascitic fluid of aE11 (produced in our laboratory) diluted 1:10000. Plasma samples were diluted 1:5, and the standard was diluted 2-fold from 1:200 to 1:12800. Terminal complement complex was detected by the monoclonal antibody 9C4 reacting with C6 (produced in our laboratory),12 which was biotinylated and diluted to 1:1000. The conjugate was streptavidin peroxidase (Amersham) diluted 1:1000.

STATISTICAL ANALYSES

The Mann-Whitney U test was used to compare the median levels of C3bc and TCC between the patient and the control group. A P value of less than .05 was regarded as significant.

The r correlation coefficients between the levels of acute hearing loss and the levels of C3bc and TCC were estimated.

RESULTS

The distribution of C3bc levels for both study patients and control subjects is shown in Figure 1.

The median value of C3bc levels was higher in the patient group (8 U/mL) than in the control group (6 U/mL) (P < .001). Even after the exclusion of 2 extreme values of 33 and 54 U/mL from the patient group, the difference remained statistically significant (P = .003). The 2 subjects with notably high levels of C3bc were both examined within 1 day after the onset of hearing loss. The correlation coefficient between the levels of acute hearing loss and the C3bc values was 0. The correlation coefficient between the levels of acute hearing loss and the TCC values was 0.

The distribution of TCC levels for both the study group and control individuals is shown in Figure 2.
The median value of TCC levels for the study group was 0.8 and that of the control group was 0.8. There was no significant difference between the median values of the 2 groups ($P = .78$).

**COMMENT**

The complement system is an important part of the host defense, mainly functioning to protect against invading microbes. However, research in recent years has focused on complement as a general mediator of tissue damage not necessarily related to infection or immune complex diseases. Thus, complement activation has been shown to contribute considerably to tissue damage occurring as a result of ischemia and reperfusion, graft rejection, trauma (physical, chemical, and thermal), and complications associated with extracorporeal circulation (hemodialysis and cardiopulmonary bypass). Some of the mechanisms by which complement induces tissue damage include histamine release, capillary leak, smooth muscle contraction, chemotaxis, phagocytosis stimulation of oxidative burst, release of arachidonic acid metabolites, stimulation of cytokine production, expression of adhesion molecules on leukocytes and endothelial cells, and stimulation of platelets. The complement system is an important mediator that should be included in the study of any inflammatory-mediated host reaction.

In our study, we found an indication of activation of the first part of the complement cascade system in patients with SD, expressed as higher median values of C3bc compared with a group of healthy controls. No difference in levels of TCC was found between the groups, thus indicating a lack of activity in the last part of the cascade. Such a dissociation between activation of the early and late part of the cascade may have several explanations. A selective activation of C3 without activation of the terminal pathway is known to occur in certain patients with nephritic factor and glomerulonephritis. The dissociation can also be explained by the different half-lives of activation products such as C3bc and TCC.

There was no correlation between the levels of hearing loss and the C3bc and TCC values. The 2 subjects with the highest C3bc values (33 and 54 U/mL) had hearing loss that lasted for less than 24 hours, which may indicate an acute activation that gradually ceased. The half-lives of the C3 activation products range from 4 to 6 hours, whereas that for TCC is just under 1 hour.

This may illustrate the importance of early blood sampling when the activation of complement is limited in time. An acute and relatively rapid attenuation of complement activation in patients with SD is consistent with our findings of slightly elevated C3bc values and normal TCC values in samples obtained several days after the onset of symptoms.

The increased levels of C3bc in plasma are unlikely to be the consequence of an inflammatory reaction taking place solely in a small organ such as the inner ear. One may speculate whether the complement activation expresses a systemic inflammatory reaction in which the symptoms from the inner ear are the only clinical symptoms expressed. In systemic inflammatory reactions, there may be other subtle symptoms of inflammation overlooked by the clinician. Complement activation might indicate a viremia, an autoimmune reaction, or some other systemic inflammatory reaction.

To our knowledge, this is the first study of complement activation in patients with SD. We aimed to find a laboratory parameter that could identify incidences of SD caused by an inflammatory and presumably autoimmune disease.

The sensitive testing of the C3 activation products described in this article may be of help in identifying patients with SD who could benefit from treatment with anti-inflammatory drugs.

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**Figure 1.** Activation in the early part of the complement cascade measured by C3bc levels in 25 patients with sudden deafness and in 24 healthy controls.

**Figure 2.** Activation in the late part of the complement cascade measured by terminal complement complex levels in 25 patients with sudden deafness and in 24 healthy controls.
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REFERENCES


Error in Terminology. In the clinical note titled “Oral Malignant Melanoma Treated With Mohs Micrographic Surgery by Fixed-Tissue Technique,” published in the February issue of the ARCHIVES (1998;124:199-201), an incorrect term was used. On page 200 in the third column, hyperfractionated regimen should have read hypofractionated regimen, 30 Gy total.