The Laryngoscope
Lippincott Williams & Wilkins, Inc., Philadelphia

Anti-Endothelial Autoantibodies in Patients With Sudden Hearing Loss

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Objectives/Hypothesis: **Sudden hearing loss (HL) can be caused by autoimmune disorders localized to the inner ear or secondary to systemic immune diseases. Studies in autoimmune animal strains showing HL have reported changes in the cochlear stria vascularis. The authors investigated the presence of antiendothelial cell antibodies (AECA) to see if immune-mediated vasculitis may play a role in human sudden HL.** ***Study Design:*** **A prospective study in patients with sudden HL.** ***Methods:*** **Fifteen consecutive patients (mean age, 32 y) affected by sudden HL and 14 normal subjects were included. Patients with familial deafness and metabolic diseases were excluded. Extensive audiovestibular, imaging, microbiological, immunological, and routine examinations were performed. AECA were detected on rat kidney tissue sections on the sera collected at −20°C.** ***Results:*** **AECA were positive in 8 of 15 patients (53%) (2 of 5 men and 6 of 10 women), thus differing significantly from the normal control population, in which only 2 of 14 tested AECA positive (P = .023).** ***Conclusions:*** **In patients with sudden HL, immune-mediated vascular damage can have a pathogenetic role and AECA might represent a serological marker of vasculitis.**
Key Words: **Sudden hearing loss, immune-mediated vascular damage, anti-endothelial cell antibodies.**
Laryngoscope, **109:1084–1087, 1999**

INTRODUCTION

Sudden hearing loss (HL) is defined as a sensorineural HL of 30 dB or more over at least three contiguous audiometric frequencies that develops over a period of a few hours to 3 days[1] and whose etiology can be found only in 10% to 15% of patients.[2] McCabe[3] reported in 1979 that a group of patients with HL responded well to immunosuppressive treatment with steroids and cyclophosphamide and described autoimmune inner ear disease as a clinical entity. Recently there has been considerable investigation into the relationship between immune system and sensorineural HL. Harris[4] demonstrated the inner ear's capacity to respond to local antigenic challenge and to produce systemic immunization. Local and systemic immunization was documented experimentally after administration of nonspecific[4–6] or inner-ear-specific antigens.[7,8]

Several authors have described a significant increase of specific HLA class II genes,[9] elevated levels of circulating immune complexes[10,11] and the detection of inner ear autoantibodies in patients with progressive or sudden sensorineural HL.[12–14] These data are consistent with an immune-mediated hypothesis of sudden HL. Nevertheless, the existence of autoimmune sudden HL has not been demonstrated yet and the response to immunosuppressive treatment cannot be used as a diagnostic criterion.

Evidence also indicates that sensorineural HL can be a manifestation of systemic autoimmune diseases[15] such as Cogan's syndrome,[16] temporal arteritis,[17] Wegener's granulomatosis,[18] polyarteritis nodosa,[19] and systemic lupus erythematosus.[20] Although the vascular damage frequently observed in lupus erythematosus and other vasculitides is probably mediated by immune complexes,[21] autoantibodies against endothelial cells (AECA) have also been implicated in this process and could have a pathogenetic role.[22] Indeed AECA may damage endothelial cells via a complement-mediated or antibody-dependent cellular cytotoxic mechanism, or by upregulation of adhesion molecules and increased secretion of proinflammatory and chemoattractant cytokines.[22] The aim of this paper is to verify if a vascular damage could have a pathogenetic role and if AECA might represent a serological marker of vasculitis in patients affected by idiopathic sudden HL.

PATIENTS AND METHODS

Fifteen consecutive patients (mean age, 32 y; age range, 14–50 y; 10 females, 5 males) affected by sudden HL were included. Patients with familial deafness and metabolic diseases were excluded. Table I shows the various types of HL. All patients underwent a routine general physical examination. Extensive audiovestibular (pure-tone average, speech discrimination, impedance audiometry, auditory brainstem response, electronystagmogram, computed dynamic posturography), imaging (magnetic resonance imaging, epi-aortic-vessels doppler ultrasound), micro-

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Editor's Note: This Manuscript was accepted for publication March 31, 1999.

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TABLE I.
Clinical Characteristics and Laboratory Data of 15 Patients With Sudden Hearing Loss.

Patient No.	Flat Loss	High-Tone Loss	U-Shaped Loss	Low-Tone Loss	ESR	CRP	CIC	ACA	ANA	Anti-DNA ds	AECA
1	Severe (bilateral)	—	—	—	High	High	Normal	–	–	–	+
2	Severe	—	—	—	Normal	Normal	Normal	–	–	–	+
3	Severe	—	—	—	Normal	Normal	Normal	–	+	–	–
4	—	Severe	—	—	Normal	Normal	Normal	–	NA	–	+
5	—	Severe	—	—	Normal	Normal	Elevated	–	+	–	+
6	—	Moderate	—	—	Normal	Normal	Elevated	–	+	–	+
7	Moderate	—	—	—	Normal	Normal	Normal	–	–	–	–
8	—	—	—	Moderate	Normal	Normal	Normal	–	–	–	+
9	—	—	—	Moderate	Normal	Normal	Normal	–	–	–	–
10	—	—	—	Moderate	Normal	High	Elevated	–	+	–	–
11	—	—	Moderate	—	Normal	Normal	Elevated	–	+	–	–
12	—	—	Moderate	—	Normal	Normal	Normal	–	–	–	–
13	Mild	—	—	—	Normal	Normal	Elevated	–	–	–	+
14	—	—	—	Mild	Normal	Normal	Elevated	–	+	+	–
15	Severe	—	—	—	Normal	Normal	Normal	–	–	–	+

CPR = C reactive protein; ESR = erythrosedimentation rate; CIC = circulating immunocomplexes; ANA = antinuclear antibodies; AECA = antiendothelial cell antibodies; ACA = anticardiolipin antibodies; NA = not available.

biological (herpesvirus, cytomegalovirus, influenza and parainfluenza virus, Epstein-Barr virus, coxsackie virus, hepatitis B and C virus, Venereal Disease Research Laboratory), immunological (antinuclear, anti-DNA double stranded, anticardiolipin, antiendothelial cell antibodies, immunocomplexes), and routine examination (blood hemoglobin and leukocyte count, erythrocyte sedimentation rate, serum gamma-globulin, C reactive protein) work-ups were performed.

Median follow-up was 4 months (range, 2–7 mo). A 1-year follow-up for AECA detection has been established. Fourteen normal subjects (7 men and 7 women; mean age, 29 y; age range, 17–45 y) without history of HL or autoimmune or metabolic diseases were included as controls.

Sera were drawn from 15 patients and from 14 control subjects and collected at −20°C. All samples of blood were obtained 1 day after the date of hospitalization and 3 days or less after the onset of sudden HL.

The AECA detection was performed as described by Tan and Pearson[23] and is based on the principle of indirect fluorescent antibody technique. The specific antibodies were detected on rat kidney tissue sections, which were coated onto the slides as monolayer (Biogenetics, Padua, Italy). In the first step, the human serum, diluted at 1:20 with PBS buffer solution, was brought into contact with the antigen substrate. Negative and positive human controls were provided with the kit (Biogenetics). The antibody, present in the test serum, attached to the antigen to form an antigen-antibody complex. Unbound material was removed by washing. In the next step, the antigen-antibody complexes were marked by specific antihuman polyvalent globulin conjugated to fluorescein (FITC). The positive reaction was shown by a green fluorescence peritubular vessel in kidney sections and observed in inverted fluorescence microscope. χ^2 test (StatView 4.0-Macintosh) was performed to compare the patient and control groups.

RESULTS

Sudden HL was unilateral in 14 cases; only one case came to our observation with a bilateral HL, which was flat and severe. In unilateral cases, HL was severe in five cases, moderate in seven, and mild in two. No characteristic shape was detected, but U-shaped and low-tone losses were more frequently associated with less severe HL (Table I). Audiological tests showed the presence of recruitment and the absence of pathological adaptation in all cases. A retrocochlear lesion was excluded by means of impedance tests and auditory brainstem response when possible (auditory threshold) and by imaging techniques. Normal vestibular reflectivity was present in all patients.

Anticardiolipin autoantibodies were negative in all patients. Antinuclear autoantibodies were positive in 6 of 14 patients (one missing) (42.8%). AECA were positive in 8 of 15 patients (53%) (2 of 5 males and 6 of 10 females), thus significantly differing from the normal population, in which only 2 of 14 tested AECA positive ($P = .023$). Although the number of patients was small, we did observe that severe hearing losses tended to be associated with positive AECA (5 of 6), whereas mild and moderate hearing losses were more frequently associated with negative AECA (6 of 9).

Titers examined for IgM antivirus were negative in all patients. The relationship among AECA and other parameters (immunological or otherwise) is showed in Table I. After corticosteroid immunosuppressive therapy (methylprednisone 1 mg/kg per day for 1 mo) no AECA-positive patients had a significant recovery, but they did show a reduction of inflammatory parameters (erythrocyte sedimentation rate, C reactive protein) without worsening of hearing or new symptoms.

DISCUSSION

The evidence in humans of coexisting sensorineural HL and systemic autoimmune disease has suggested the

possibility of isolated forms of immune-mediated deafness. The results of treatment of individual cases with immunosuppressive agents also support the hypothesis that some cases of so-called "idiopathic" sudden HL are immune-mediated.[12,24] Response to immunosuppressive treatment can be demonstrated by a reduction of inflammatory parameters and by the absence of other systemic or local manifestations and not only by a recovery of hearing. Moreover, long-term studies are necessary to determine if autoimmune sudden HL is an initial local manifestation of a systemic autoimmune disease or if it is an isolated entity.

This is the first report of AECA in sudden HL. The pathogenetic potential of AECA has been demonstrated in vascular diseases, especially for immune-mediated vasculitis.[25] For this reason, and because AECA were present in 53% of our patients (8/15), we suspect that AECA induced vascular damage of the inner ear in these cases.

In fact, AECA, reacting against available surface antigens, may damage endothelial cells via a complement-mediated or antibody-dependent cellular cytotoxic mechanism, or by upregulation of adhesion molecules. Although AECA do not display any disease specificity, their absence in diseases such as mixed essential cryoglobulinemia, in which vascular damage is clearly mediated by other immune effectors, suggests that these antibodies represent a primary event rather than merely a secondary immune response against antigens exposed in the course of the vascular inflammatory process.[22]

The hypothesis that vascular damage might have a pathogenetic role in immune-mediated sudden HL is supported by labyrinthine fibrosis and cochlear ossification found in patients affected by autoimmune diseases and sudden HL,[26] and by the observation of the breakdown of stria vascularis blood-labyrinth barrier in C3H/lpr autoimmune disease mice that have elevated auditory brainstem response thresholds during the active phase of the disease. Some researchers suggest that either a humoral mechanism of injury or a direct injury to the endothelial cells is involved and that the breakdown of tight junctions between stria vascularis endothelial cells may be one etiologic mechanism of sensorineural HL in autoimmune disease.[27]

In our AECA-positive patients, the absence of HL recovery after steroid therapy might be explained by irreversible vascular damage of the inner ear, as described in patients with sudden HL and systemic vasculitis.[26] Therefore, the immunosuppressive treatment cannot be utilized as the only diagnostic criterion.

The finding in our patients of antinuclear antibodies (42.8%) and circulating immunocomplexes (40%) also supports the involvement of immune mechanisms in this disease and is consistent with the experimental model of autoimmune inner ear disease of C3H/lpr mouse where significantly elevated threshold shifts correlated with development of antinuclear antibodies and circulating immunocomplexes.[28]

A systemic disease should produce bilateral rather than unilateral ear symptoms over time. In fact, unilateral symptoms more often result from primary ear disease (e.g., eighth nerve schwannoma and chronic otitis media), but, as in ocular autoimmune diseases,[29,30] the microvascular damage may be unilateral. Moreover, the finding of AECA supports a vascular hypothesis and should encourage investigation about the existence of an immune systemic disease.

The preponderance of women in our group of patients with sudden HL is noted in other autoimmune diseases. This link to the female sex may be influenced by endocrine effects on the immune system, but the cause remains unclear.

CONCLUSION

The present data suggest an immunological mechanism in the pathogenesis of some cases of idiopathic sudden HL. Detection of serological nonspecific inner-ear autoantibodies, such as AECA, in patients with sudden HL may be useful in clinical practice to identify a subset of sudden HL patients whose inner ear damage may be caused by immune-mediated vasculitis. The diagnosis of AECA-related autoimmune sudden HL should support an aggressive immunosuppressive treatment to avoid the worsening of HL or the onset of new symptoms. Further studies on a larger population are needed to support this hypothesis and to demonstrate the possible role of autoantibodies such as AECA in the development of immune-mediated sudden HL.

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