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Clinical Associations of Serum Antiendothelial Cell Antibodies in Patients With Sudden Sensorineural Hearing Loss

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Objectives/Hypothesis: **The role of antiendothelial cell antibodies in systemic vasculitis has been reported. The aim of the study was to define the clinical associations of serum antiendothelial cell antibodies in patients with sudden sensorineural hearing loss.** ***Study Design:*** **A prospective study in patients with sudden sensorineural hearing loss.** ***Methods:*** **Serum samples were taken from 59 consecutive patients with sudden sensorineural hearing loss at time of presentation and from 28 normal control subjects. Indirect immunofluorescence assay was used to detect antiendothelial cell antibodies.** ***Results:*** **The prevalence of antiendothelial cell antibody detection was 54% (32 of 59 patients), with a statistically significant difference between patients and control subjects (*P* = .0004). Antiendothelial cell antibody positivity was significantly associated with absent recovery of hearing loss (*P* = .0020).** ***Conclusions:*** **The cytotoxicity to endothelial cells of the inner ear by antiendothelial cell antibody–positive sera might play a role in causing the stria vascularis damage in immune-mediated sudden sensorineural deafness. The appearance of antiendothelial cell antibody is related to the poor outcome of hearing loss, and its detection could be helpful in the selection of particular patients with sensorineural hearing loss for specific immunosuppressive treatments.** ***Key Words:*** **Sudden sensorineural hearing loss, antiendothelial cell antibodies, inner ear, autoimmunity.**

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INTRODUCTION

Sudden hearing loss is a sensorineural hearing impairment that develops over a period of a few hours to a few days. The incidence of sudden sensorineural hearing loss (SSHL) has been reported to range from 5 to 20 cases per 100,000 persons per year.

Several theories have been proposed regarding the development of SSHL. There is considerable evidence suggesting that hearing can be influenced by immunity in the inner ear. Immunity can protect against infections of the labyrinth, but immune response may also damage the delicate tissues of the inner ear. Antigenic challenge of the inner ear of sensitized animals leads to rapid accumulation of leukocytes, antibody production, hearing loss, and tissue damage. Moreover, a number of systemic autoimmune disorders include hearing loss and vertigo as part of their constellation of symptoms, such as systemic lupus erythematosus, Cogan syndrome, Wegener's granulomatosis, relapsing polychondritis, polyarteritis nodosa, Sjögren syndrome, temporal arteritis, and delayed contralateral endolymphatic hydrops.[1]

It also appears that autoimmune damage can exist as an entity confined to the labyrinth. The pathogenesis of autoimmune hearing loss includes vasculitis of inner ear vessels, cross-reacting antibodies, or autoantibodies directed against inner ear antigenic epitopes. In patients with SSHL, serological autoantibodies against specific and non–organ-specific antigens of the inner ear (types II and IX collagen,[2,3] P30[4] and P80 cochlear proteins,[5] cardiolipin,[6] and phospholipids, serotonin, and ganglioside[7]) have been found, and a reduction of T lymphocyte subpopulations C3, C4, and C8,[8,9] together with increased levels of the C3bc complement factor,[10] has been detected.

The clinical presentation of immune-mediated sensorineural hearing loss can be highly variable. It is extremely important to recognize immune disorders of the inner ear because they are among the few forms of hearing loss that are currently amenable to medical treatment. In addition, recent developments in understanding the intracellular pathways that participate in damage to the inner ear provide new opportunities for pharmacotherapy of immune-mediated disorders of hearing.

Antiendothelial cell antibodies (AECAs) are a heterogeneous group of antibodies directed against a variety of antigenic determinants on endothelial cells, and they have

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been detected in several diseases that share a varying degree of vessel wall damage. There is increasing evidence that AECAs might be pathogenic in inducing autoimmune vascular disease. It is relevant to note that the presence of AECAs was correlated with disease activity in systemic vasculitis. In fact, experimental in vitro and in vivo models have supported a potential pathogenic role for AECAs in sustaining immune-mediated vessel damage.[11] We previously reported the presence of serum antiendothelial cell autoantibodies in patients with SSHL.[12] The aim of the present study was to define the associations between serum AECAs, clinical manifestations, and response to steroid immunosuppressive therapy in patients with SSHL.

PATIENTS AND METHODS

Patients

Fifty-nine consecutive patients (32 female and 27 male patients; age range, 13–68 y [mean age, 39 y]) affected by SSHL were included in the study. Patients with familial deafness and metabolic diseases were excluded. All patients underwent a routine general physical examination. Pure-tone audiometry, speech discrimination test, impedance audiometry, auditory brainstem response testing, electronystagmogram, and computed dynamic posturography and imaging (magnetic resonance imaging, epiaortic vessels ultrasound) were performed in all patients. The degree and type of hearing loss and the clinical characteristics of the patients are shown in Table I. We used the following scale of hearing loss degree: mild, >20–≤40 dB hearing loss; moderate, >40–≤70 dB hearing loss; severe, >70–≤90 dB hearing loss; and deep, >90 dB hearing loss. The average pure-tone hearing level threshold for five frequencies (0.25–4 kHz) was analyzed for each patient. Virological and microbiological test results for herpes, cytomegalovirus, influenza and parainfluenza, Epstein-Barr virus, Coxsackie virus, hepatitis B and C viruses infection, and sexually transmitted disease were examined. An immunological evaluation was performed by the detection of antinuclear, anti-DNA double-stranded, anticardiolipin antibodies, AECAs, immunocomplexes in the sera of patients. Blood hemoglobin and leukocyte counts, erythrocyte sedimentation rate, and serum γ-globulin and C-reactive protein values were obtained. Twenty-eight normal subjects (14 male and 14 female subjects; age range, 17–45 y [mean age, 29 y]) without history of hearing loss or autoimmune or metabolic diseases were included as control subjects. Antiendothelial cell antibody (AECA)–positive patients with shearing loss gave their consent to have a mono-drug therapy with steroids. Antiendothelial cell antibody–positive patients were treated with 1 mg/kg methylprednisolone daily for 1 month, and AECA–negative patients were treated according to our protocol for idiopathic sudden hearing loss, which consists of a combined regimen of steroids (1 mg/kg methylprednisolone daily) for 15 d, plasma expander (500 mL/d low-molecular-weight dextran) for 5 d, and 100 mg/d acetyl salicylic acid for 15 days. The average hearing recovery for five frequencies (0.25–4 kHz) was analyzed in each subject 1 month after treatment and every 3 months; median follow-up was 18 months (range, 9–29 mo). An average hearing level improvement of more than 20 dB was classified as "good recovery," and improvement between 10 and 20 dB as "fair recovery." Changes in hearing threshold within 10 dB were judged to be equivalent to no change. When hearing level returned to normal, recovery was defined as "returned to predisease levels."

Antiendothelial Cell Antibody Detection

Serum samples were drawn from 59 patients and 28 control subjects and stored 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 SSHL. The AECA detection was performed as described by Tan and Pearson[13] by using indirect fluorescent antibody technique. The specific antibodies were detected on rat kidney tissue sections, which were coated onto slides as monolayers (Biogenetics, Padua, Italy). The human serum, diluted at 1:20 with phosphate-buffered saline, was brought into contact with the antigen substrate. Negative and positive human controls were provided with the kit (Biogenetics). The antibody, present in the serum, reacted with the antigens forming an antigen-antibody complex. Unbound material was removed by washing. The antigen-antibody complexes were marked with specific anti-human polyvalent globulin conjugated to fluorescein (fluorescein isothiocyanate). The positive reaction was shown by a green fluorescence of the peritubular vessels in the kidney sections and observed in the inverted fluorescence microscope.

TABLE I.
Distribution of AECA Presence According to Clinical Characteristics in 59 Sensorineural Sudden Hearing Loss Patients.

	No.	AECA + (%)	*P**
Total			
Patients	59	32 (52%)	
Control subjects	28	4 (14%)	.0004
Sex			
Females	32	20 (62%)	
Males	27	12 (44%)	.1970
Hearing loss type			
Flat	31	18 (58%)	
U-shaped	4	3 (75%)	
High frequency	10	6 (67%)	
Low frequency	14	5 (36%)	.6060†
Hearing loss degree			
Deep	6	4 (80%)	
Severe	24	13 (57%)	
Moderate	19	9 (53%)	
Mild	10	6 (67%)	.7961‡
Hearing recovery			
Absent	17	15 (88%)	
Fair	9	4 (44%)	
Good	18	6 (33%)	
Returned to predisease levels	15	7 (47%)	.0020§

*P value: Fisher exact two-tailed P from 2 × 2 contingency table.
†P value obtained considering flat hearing loss versus the other hearing loss types.
‡P value obtained considering deep and severe hearing loss versus moderate and mild hearing loss.
§P value obtained considering absent versus fair, good, and returned to predisease levels recovery.

Statistical Analysis

Fisher's Exact test (Stat View version 4.0, Macintosh) was performed to compare data from patients and control subjects and to analyze distribution of AECA detection according to clinical parameters. Fisher's Exact two-tailed P value was obtained from 2 × 2 contingency tables.

RESULTS

Antiendothelial cell antibody positivity was detected in 32 of 59 patients (52%) and in 4 of 28 control subjects

TABLE II.
Correlation Between Hearing Outcome, Degree and Type of Hearing Loss and AECA Detection in 59 Sensorineural Sudden Hearing Loss Patients.

	Absent Hearing Recovery No.	Present Hearing Recovery No.	P*
AECA detection			
AECA +	15	17	.0012
AECA −	2	25	
Hearing degree			
Deep and severe	9	21	1
Mild and moderate	8	21	
Type of hearing loss			
Flat HL	12	19	.0924
HHL, LHL, U-SHL	5	23	

*P value: Fisher exact two-tailed P from 2 × 2 contingency table.

(14%), differing in a statistically significant manner ($P = .0004$). Distribution of AECA presence and clinical characteristics of 59 patients with SSHL are shown in Table I.

No statistically significant associations between AECA positivity and sex, hearing loss type, and hearing loss degree were observed. The results of hearing recovery are compared with the audiometric examination after 1 month of therapy. During the follow-up period all patients did not show any changes of hearing recovery. An absent hearing recovery was significantly associated with AECA presence (P = .0020).

In Table II, the association between hearing outcome and hearing loss degree, hearing loss type, and AECA presence is reported. Only AECA presence correlated to a poor outcome in a statistically significant way (P = .0012); no relation was noted with hearing loss degree and hearing loss type.

Figure 1 shows the pretreatment and post-treatment audiograms relative to two of 59 patients with SSHL in which both serum AECA presence and anticardiolipin antibody presence were detected. Blood hemoglobin and leukocyte count, erythrocyte sedimentation rate, serum γ-globulin values, and C-reactive protein examination results were not statistically different between AECA–positive and AECA–negative patients. No statistically signif-

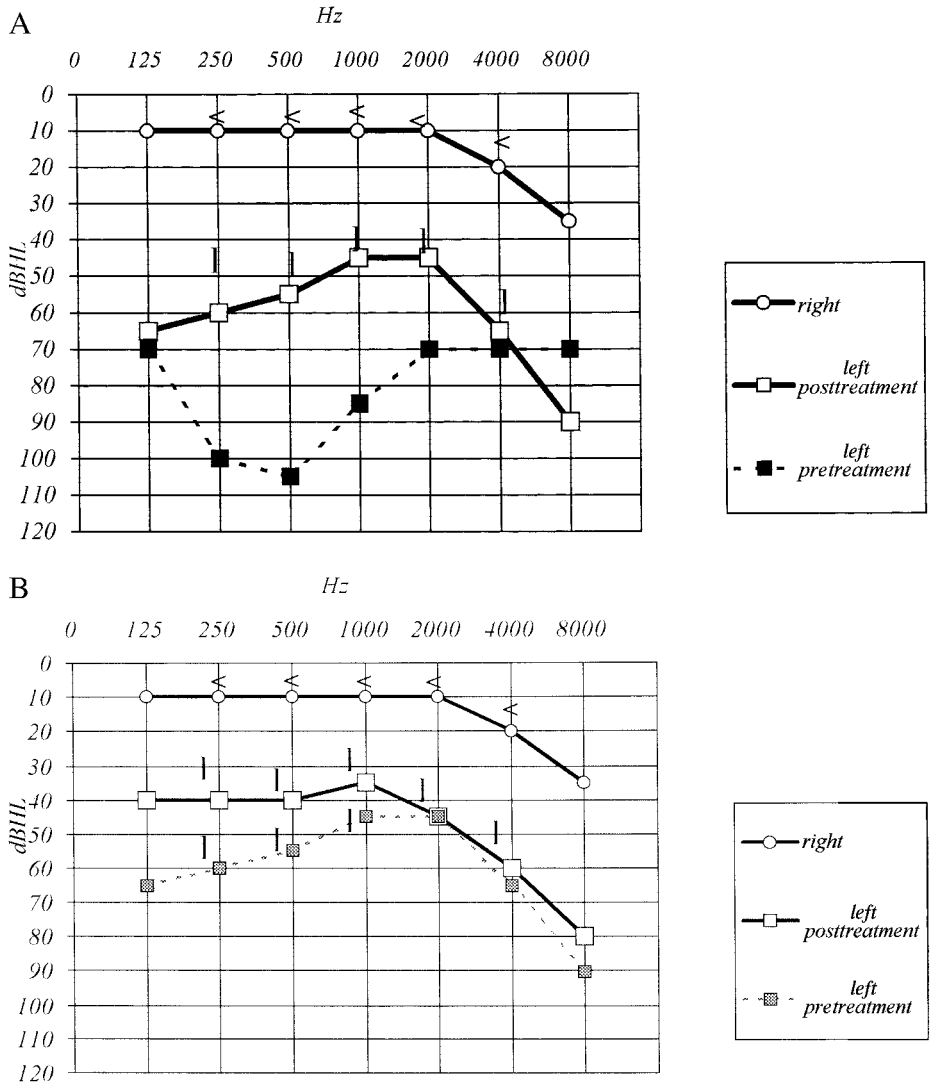

Fig. 1. (**A** and **B**) The audiograms relative to two antiendothelial cell antibody–positive and anticardiolipin antibody–positive patients with left sudden sensorineural hearing loss, showing hearing loss degree and type before and after immunosuppressive steroid treatment.

icant association between antinuclear, anti-DNA double-stranded, anticardiolipin antibodies, AECA, immunocomplexes and AECA positivity has been observed. Results of virological and microbiological tests for herpes, cytomegalovirus, influenza and parainfluenza, Epstein-Barr virus, Coxsackie virus, hepatitis B and C viruses infection, and sexually transmitted disease were negative in all patients.

DISCUSSION

Antiendothelial cell antibodies have been described in a variety of diseases including connective tissue disorders and systemic vasculitis.[11,14–16] The present data confirm our previous observation of AECA presence in a subset of patients with idiopathic SSHL.[2] Furthermore, the statistically significant association between AECA presence and hearing loss in a larger population of patients with idiopathic SSHL supports the hypothesis that AECAs could play a role in the onset of a subset of cases of immune-mediated sensorineural hearing loss.

How AECAs are able to induce inner ear vascular damage is still matter of discussion. The intimate mechanism by which endothelial cell injury is induced during immune-mediated sensorineural hearing loss could be due to a breakdown of tight junctions between stria vascularis endothelial cells,[17] which leads to alterations of stria blood–labyrinth barrier and decreased endocochlear potentials.[18] Antiendothelial cell antibodies could induce stria vascularis endothelial cell injury by different mechanisms such as complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and inducing upregulation in the expression of endothelial adhesion molecules and/or secretion of chemoattractants and cytokines.

Further studies are necessary to elucidate the mechanisms by which AECAs injure stria vascularis endothelial cells and to characterize inner ear target antigens, to clarify the possible pathophysiological role of these antibodies in vascular immune-mediated SSHL or to establish whether they represent an epiphenomenon rather than the only cause of SSHL. Although AECAs 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 antigenes exposed in the course of the vascular inflammatory process.[16]

The most important prognostic factors in sudden hearing loss are considered to be a delayed treatment and the degree of deafness. In our series, the severity of hearing loss at time of presentation and type of deafness was not associated with a poor outcome, in opposition to other reports.[19,20] Instead, the AECA presence seems to be a marker of poor prognosis. In fact, 15 of 17 patients (88%) who had an absent hearing recovery were AECA positive, and the association between AECA presence and poor outcome was statistically significant. The favorable response to steroid treatment in 17 of 32 AECA-positive patients with SSHL could imply that, in those cases, irreversible injury of the inner ear had not yet occurred, in contrast to what happened in the 15 AECA-positive patients who had an absent hearing recovery.

CONCLUSION

Autoimmune inner ear disease is a treatable cause of SSHL, and it is important for physicians and hearing health professionals to recognize that proper early diagnosis and management strategies may result in stabilization of and, possibly, improvement in hearing. Inexpensive and feasible tests such as serological AECA detection could be useful in the differential diagnosis of vascular immune-mediated SSHL. More particularly, the appearance of AECAs seems to be related to the poor outcome of hearing loss, and its detection could be helpful in selecting particular patients with SSHL for specific immunosuppressive treatments. Further studies are needed to better clarify the role of AECA presence in SSHL. Antiendothelial cell antibody–positive patients with SSHL must be followed to evaluate the clinical outcome, the onset of systemic immunological disorders, and the evolution of hearing loss and, consequently, to elucidate the clinical role of AECA detection in idiopathic sudden hearing loss.

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