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Autoimmunity in Sudden Sensorineural Hearing Loss: Possible Role of Anti-endothelial Cell Autoantibodies*

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In order to verify whether anti-endothelial cell autoantibodies (AECAs) can be used as serological markers of inner ear vasculitis in sudden sensorineural hearing loss (SSHL), 32 patients affected by idiopathic SSHL were investigated. All patients underwent a routine general physical examination and extensive audiovestibular, microbiological and immunological investigations. Fourteen normal subjects without a history of HL, autoimmune or metabolic disease served as controls. Detection of AECAs was performed using an indirect immunofluorescence technique. AECA-positive patients were treated with methylprednisone, while AECA-negative patients were treated with a combined regimen of steroids, plasma expander and aspirin. The average hearing recovery for 5 frequencies (0.25–4 kHz) was analyzed in each subject 1 month after treatment and every 3 months thereafter; median follow-up was 12 months (range 9–18 months). A total of 15/32 patients (46.8%; 11/19 females, 4/13 males) were AECA-positive and thus differed significantly from the normal population in whom only 2/14 tested cases were positive ($p = 0.03$). Severe hearing loss was associated with being AECA-positive in 8/11 cases. During follow-up, 25/32 patients improved their hearing and 17 of these patients were AECA-negative. The seven cases without hearing improvement were all AECA-positive. In patients with SSHL, immune-mediated vascular damage may have a pathogenetic role and AECAs may represent a serological marker of vasculitis even if they are not inner ear-specific and even if they represent an epi-phenomenon rather than the only cause of SSHL. *Key words: anti-endothelial cell autoantibodies, immune-mediated hearing loss, sensorineural sudden hearing loss.*

INTRODUCTION

Sudden sensorineural hearing loss (SSHL) is defined as a hearing loss of ≥ 30 dB over at least 3 contiguous audiometric frequencies that develops over a period of a few hours to 3 days (1) and whose etiology can be found in only 10–15% of patients (2). The main known causes of SSHL have been classified as infectious, traumatic, neoplastic, toxic, circulatory, neurologic and metabolic. Other identified causes of SSHL are Ménière's disease and genetic predisposition (3). When no cause can be identified SSHL is described as idiopathic.

Some cases of sudden deafness, Ménière's disease and rapidly progressive sensorineural hearing loss can be included in a heterogeneous group of diseases known as "autoimmune inner ear diseases" (4, 5) or immune-mediated inner ear disease (IMIED) (6). IMIED can be either primary and localized to the inner ear or secondary to generalized systemic immune diseases such as systemic lupus erythematosus, Cogan's syndrome, Wegener's granulomatosis, relapsing polychondritis, polyartheritis nodosa, Sjogren's syndrome, temporal arteritis and delayed contralateral endolymphatic hydrops (6).

In SSHL serologic autoantibodies against specific and non organ-specific antigens of the inner ear [type II and type IX collagen (7, 8); P30 (9) and P80 cochlear proteins (10); cardiolipin (11), phospholipids, serotonin and ganglioside (12)] have been found and a reduction in T-lymphocyte subpopulations C3, C4 and C8 (13, 14), together with increased levels of the C3bc complement factor (15), has been detected.

Anti-endothelial cell autoantibodies (AECAs) have been described in a variety of different diseases, including connective tissue disorders (systemic lupus erythematosus, scleroderma, rheumatoid arthritis with vasculitis, dermatomyositis, mixed connective disease), systemic vasculitis (Wegener's granulomatosis, microscopic polyangitis, Kawasaki's disease, Beçhet's disease, Takayasu's arteritis, idiopatic retinal vasculitis) and other diseases such as hemolytic uremic syndrome, multiple sclerosis and diabetes mellitus. AECAs represent a heterogeneous group of antibodies directed against a variety of antigen determinants on endothelial cells that are detected in various inflammatory disorders (16). They can interfere with several endothelial cell functions and may therefore be of pathophysiological relevance. These autoantibodies react against available surface antigens and may damage endothelial cells via a complement-mediated or antibody-dependent cellular cytotoxic mechanism or by upregulation of adhesion molecules.

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The aim of the present paper is to verify whether AECAs may represent a serologic marker of vasculitis in a group of patients affected by idiopathic SSHL.

MATERIALS AND METHODS

Patients

Thirty-two consecutive patients (19 females, 13 males; mean age 33 years; range 14–60 years) 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 and pure-tone audiometry, speech discrimination test, impedance audiometry, auditory brainstem responses (ABRs), electronystagmogram, computed dynamic posturography and imaging studies (MRI, epi-aortic-vessels ultrasound) were performed in all patients.

Virological and microbiological tests for herpesvirus, cytomegalovirus, influenza and parainfluenza, Epstein–Barr virus, Coxsackie virus, hepatitis B and C viruses and venereal disease were conducted. An immunological evaluation was performed by the detection of antinuclear, antiDNA double-stranded, anticardiolipin, antiendothelial cell antibodies and immunocomplexes in patient sera. Determination of blood hemoglobin and leukocyte counts, erythrocyte sedimentation rate and serum gamma-globulin and C-reactive protein levels was performed.

Fourteen normal subjects (7 males, 7 females; mean age 29 years, range 17–45 years) without a history of HL, autoimmune or metabolic disease were included as controls. AECA-positive patients were treated with methylprednisone (1 mg/kg/day for 1 month), while AECA-negative patients were treated with a combined regimen of steroids (methylprednisone 1 mg/kg/day for 15 days), plasma expander (low-molecular-weight dextran 500 ml/day for 5 days) and aspirin (100 mg/day for 15 days). The average hearing recovery for 5 frequencies (0.25–4 kHz) was analyzed in each subject 1 month after treatment and every 3 months thereafter; median follow-up was 12 months (range 9–18 months). An average hearing level improvement of >20 dB was classified as "good recovery", improvement of 10–20 dB as "fair recovery", changes in hearing threshold of ≤ 10 dB were judged to be equivalent to no change and when hearing level returned to normal, recovery was defined as "complete".

AECA detection

Sera were drawn from 32 patients and 14 controls and stored at -20°C. All blood samples were obtained 1 day after hospitalization and ≤ 3 days after the onset of SSHL. AECA detection was performed as described by Tan and Pearson (17) using an indirect fluorescence 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 1 : 20 with phosphate-buffered saline buffer, was first 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 to form an antigen–antibody complex. Unbound material was removed by washing. The antigen–antibody complexes were next marked with specific antihuman polyvalent globulin conjugated to fluorescein isothiocyanate. A positive reaction was indicated by green fluorescence of the peritubular vessels in the kidney sections and observed in an inverted fluorescence microscope. A χ^2 test (Stat View 4.0, Macintosh) was performed to compare data from patients and controls.

RESULTS

SSHL was unilateral in 30 patients and bilateral in 2. Of the unilateral cases, SSHL was severe in 11 patients, moderate in 14 and mild in 5; both bilateral cases presented with severe SSHL. No characteristic shape was detected: 19 patients had flat SSHL, 5 high-frequency SSHL, 2 U-shaped SSHL and 6 low-frequency SSHL. Moreover, U-shaped and low-frequency SSHLs were more frequently associated with less severe HL. Recruitment was present and pathological adaptation absent in all cases. A retrocochlear lesion was excluded by means of impedance tests and ABR where possible (depending on auditory threshold) and by imaging techniques. Normal vestibular reflectivity was present in all patients. Anticardiolipin autoantibodies were negative in all patients (data not available for 1 patient); antinuclear autoantibodies were positive in 10 patients (data not available for 1 patient); anti-DNA double-stranded autoantibodies were negative in all except 1 patient (data not available for 2 patients); and circulating immunocomplexes were positive in 11 cases (data not available for 1 patient) (Table I).

Fifteen out of 32 patients (46.8%; 11/19 females, 4/13 males) were AECA-positive and thus differed significantly from the normal population in whom only 2/14 tested cases were AECA-positive ($p = 0.03$). Although the number of patients was small, we observed that severe hearing loss was associated with AECA positivity in 8/13 cases. During follow-up an improvement in hearing was detected in 25/32 patients: in 6 cases hearing threshold returned to normal, in 12 the recovery was "good" and in 7 it was "fair".

The 7 cases in whom no improvement occurred were all AECA-positive and 17/25 patients who improved were AECA-negative. After treatment all patients, even those without hearing recovery, showed a reduction in inflammatory parameters (erythrocyte sedimentation rate and C-reactive protein) and, during follow-up, clinical conditions and hearing level remained stable.

DISCUSSION

The pathogenetic potential of AECAs has been demonstrated in vascular diseases and especially in immune-mediated vasculitis (18). For this reason, and because AECAs were present in 46.8% of SSHL patients (15/32) but in only 2/14 normal subjects, we hypothesized that they can induce vascular damage of the inner ear. Although AECAs do not display any disease specificity, their absence in diseases such as mixed essential cryoglobulinemia, in which vascular damage is mediated by other immune effectors, suggests that the presence of these antibodies could represent a primary event rather than merely a secondary immune response against antigens exposed during a vascular inflammatory process (19).

The hypothesis that SSHL may be due to an immune-mediated vascular disease is supported by histopathological findings on human temporal bone specimens. In fact, in immune-mediated SSHL, labyrinthine fibrosis and ossification are the most common findings in the cochlea, supporting the theory that vascular damage may be induced by AECAs. In contrast, in idiopathic sudden hearing loss, histopathological findings mainly represented by degeneration of the spiral ligament, vascular stria, hair cells, dendrites and apical spiral ganglion cells suggest a viral etiology (20).

Table I. *Type of hearing loss in relation to immunological signs of autommunity in 32 patients with SSHL*

Patient No.	Type of hearing loss				CIC	ACA	ANA	A-DNAds	AECA
	Flat	HT	US	LT					
1	Severe				Normal	–	–	–	+
2	Severe				Normal	–	–	–	+
3	Severe				Normal	–	+	–	–
4		Severe			Normal	–	N/A	–	+
5		Severe			Elevated	–	+	–	+
6		Moderate			Elevated	–	+	–	+
7	Moderate				Normal	–	–	–	–
8				Moderate	Normal	–	–	–	–
9				Moderate	Normal	–	–	–	+
10				Moderate	Elevated	–	+	–	–
11			Moderate		Elevated	–	+	–	–
12			Moderate		Normal	–	–	–	–
13	Mild				Elevated	–	–	–	+
14				Mild	Elevated	–	+	+	–
15	Severe				Normal	–	–	–	+
16	Moderate				N/A	–	–	–	–
17		Severe			Normal	–	+	–	–
18	Severe				Elevated	N/A	+	N/A	+
19	Moderate				Normal	–	–	–	–
20	Moderate				Normal	–	–	–	+
21	Severe				Normal	–	–	–	+
22	Severe				Elevated	–	–	–	+
23	Mild				Elevated	–	–	–	+
24	Severe				Normal	–	–	–	+
25	Moderate				Normal	–	–	–	–
26		Moderate			Elevated	–	+	–	–
27	Moderate				Normal	–	–	–	–
28				Mild	Normal	–	+	–	–
29	Severe				Elevated	–	–	–	–
30				Mild	Normal	–	–	N/A	–
31	Severe				Normal	–	–	–	–
32	Mild				Normal	–	–	–	+

HT = high tone; LT = low tone; US = U-shaped; CIC = circulating immunocomplexes; ACA = anticardiolipin autoantibodies; ANA = antinuclear autoantibodies; AECA = anti-endothelial cell autoantibodies; A-DNA ds = anti-DNA double-stranded autoantibodies.

The intimate mechanism by which endothelial cells are injured during immune-mediated sensorineural hearing loss could be due to a breakdown of tight junctions between the stria vascularis endothelial cells (21).

The response to immunosuppressive therapy cannot be used as the only diagnostic criterion for SSHL. In fact, the absence of auditory threshold recovery, in spite of steroid treatment, in all seven AECA-positive patients could be explained by irreversible vascular damage to the inner ear (22). Based on our results the presence of AECAs in SSHL can be considered a negative prognostic factor, but a larger population needs to be studied in order to confirm such a hypothesis.

Although several authors have suggested that immune-mediated vasculitis should be suspected only in bilateral SSHL, immune-mediated microvascular damage may be unilateral, as in ophthalmologic immune diseases (23, 24).

In conclusion, the present data suggest that in patients with immune-mediated SSHL vascular damage may play a pathogenetic role and AECAs may represent a serological marker of vasculitis even if they are not inner ear-specific and may represent an epi-phenomenon rather than the only cause of SSHL. Further studies are needed to support this hypothesis and to determine if immune-mediated SSHL is an initial local manifestation of a systemic autoimmune disease or an isolated clinical entity.

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